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A microflow system for particle separation and analysis  
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# A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

## FIELD OF THE INVENTION

5 The present invention relates to methods and apparatuses for detection, separation and sorting of particles, such as cells, cell organelles, beads, molecules, such as Deoxyribonucleic acid (DNA), proteins, etc. in a fluid. In particular, the invention relates to particle separation by using different forces such as magnetic, electrophoretic, hydrodynamic and/or gravitational forces, e.g. for utilisation in flow cytometry, light microscopy, electrophoretic separation,  
10 magnetophoresis, etc.

## BACKGROUND OF THE INVENTION

Flow cytometry is a well known technique that is used for high throughput measurements of  
15 optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyse and isolate cells and organelles with particular physical, biochemical, and immunological properties.

Traditionally, cell sorting by flow cytometry (fluorescence activated cell sorting) has been the  
20 method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Also the equipment is very costly with high operation and maintenance cost, making the technique available only to a limited number of laboratories.

25 During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterisation of cell populations. Immunomagnetic cell separation, e.g. as commercially introduced by Dynal A/S and Miltenyi Biotec, has become an established method for cell analysis in clinical diagnostics. Due to the relatively low prize, this method is attractive in flow cytometry, especially in sorting of rare  
30 cellular events. For example, sorting of fetal cells in maternal blood provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of *chorionic villus* sampling. Another rare event scenario is the detection of low concentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of bacterial and viral  
35 diseases.

Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter analysis readily available with flow cytometry techniques. Existing techniques for magnetic  
40 separation are suffering from the low purity of the sorted cell fraction and the low recovery rate

of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which then causes cell losses. Additionally small subpopulation of labelled cells cannot be directly isolated by existing magnetic separation techniques.

- 5 A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting, (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

## SUMMARY OF THE INVENTION

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The present invention provides a micro flow system (1) for separating particles (12,13), e.g. by magnetic or electrophoretic means, comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) containing the particles (12,13) in the channel (5), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for  
15 discharging fluid from the flow channel (5) and field generating means (8) positioned proximate to the flow channel (5) for generating a field substantially across the flow channel (5) whereby particles (12) entering the flow channel (5) and being susceptible to the field generated across the flow channel (5) are deflected in the flow channel (5).

20

The present invention also provides a micro flow system (1) for separating particles (12,13) by hydrodynamic means, comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) containing the particles (12,13) in the channel (5), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for discharging fluid from the flow channel (5), a transparent or translucent cover (16) for covering the flow channel  
25 and allowing observation of events in the flow channel, wherein events in the flow channel activate controlling means (19) for controlling the flow through the second outlet means (6), and optionally through the first outlet means (7), said controlling means (19) being connected with optical detection means (27) which is adapted to detect said events.

30

The present invention furthermore provides a micro flow system (1) for analysing components of a fluid (9), comprising a member having a flow channel (5) defined therein for laminar flow of the fluid (9), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for discharging fluid (9) from the flow channel (5) and a plurality of areas (112) located in the flow channel and comprising immobilised reagents so that the fluid is analysed for a  
35 plurality of components in one operation.

40

The present invention also provides a micro flow system (1) for separating particles (12,13) by gravitational means, comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) and an acceptor buffer (10) in the channel (5), first inlet means (2) for  
entering an upper fluid (9) comprising the particles (12,13) into the flow channel (5), upper outlet

means (7) for discharging fluid from the flow channel (5), second inlet means (3) for entering an lower fluid (10), lower outlet means (6) for discarding fluid comprising particles (12), the flow of within the flow channel being adapted so that at least a part of the particles, due to the higher density relative to the upper fluid (9), are allowed to sediment into the lower fluid (10).

5

Furthermore, the present invention relates to a methods for separating particles, a method of analysing components of a fluid, and a method of forming sites comprising immobilised reagents in a flow channel.

10

Thus, it is an object of the present invention to provide a micro flow system and a method particle separation having an improved efficiency of particle separation compared to the prior art.

15

It is another object of the present invention to provide a micro flow system and a method for particle separation in which cell lysis is minimised.

It is yet another object of the present invention to provide an improved method for preparation of samples containing fluids for separation and analysis of particles.

20

It is a still further object of the present invention to provide a micro flow system and a method for simultaneous separation of particles into a plurality of groups of particles.

It is a still further object of the present invention to provide a micro flow system including facilities for pre-treatment and/or post-treatment of a sample.

25

#### DETAILED DESCRIPTION OF THE INVENTION

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The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may comprise living cells, chromosomes, organelles, biomolecules, such as proteins, etc.

35

According to an important aspect of the invention, the flow through the flow channel is a laminar flow so that flow of particles are predictable and easy to control, e.g. with a flow of guiding buffers.

When the flow is laminar, the stream of particles can be positioned as desired within the flow channel, e.g. by controlling flow velocities of the fluid containing particles at the particle inlet of the member and flow velocities of guiding buffers at corresponding inlets.

Preferably, the flow channel is small for the flow through the channel to have a low Reynolds number, e.g. in the range of 0.01-500, such as 0.05-50, preferably 0.1-25. Thereby, inertial effects, which causes turbulence and secondary flows are negligible, viscous effects dominates the dynamics, and mixing is caused only by diffusion. Flow of fluid containing particles and guiding buffers can be laminated in guided layers through the channel and displacement of particles in the channel is only caused by the force.

The illustrated flow channels of the micro flow system have a width ranging from 0.1 to 0.55 mm, preferably ranging from 0.1 to 0.4 mm, in particular ranging from 0.1 to 0.2 mm, and a depth ranging from 0.04 to 0.2 mm, preferably ranging from 0.04 to 0.1. With respect to the lowest cross-sectional area of the flow channel, it is preferred that this area is in the range of 0.004 to 0.11 mm<sup>2</sup>, in particular in the range of 0.004 to 0.02 mm<sup>2</sup>.

It is believed that any length of the flow channel within the range of 0.1 to 20 mm, preferably 1.0 to 3.5 mm, would lead to satisfactory results.

The system is operating with total volumetric flow rates of 1.0 and 200  $\mu$ l/min which gives a flow velocity of 15 mm/min up to 180 mm/min. The average residence time of a particle inside the flow channel which corresponds to the separation time ranges from 0.1 to 6 sec. The residence time of the sample is defined by the total volumetric flow rate of the system. The higher the flow rate the lower is the influence of the magnetic field on the sample flow stream.

The micro flow system may comprise flow speed adjustment means for adjustment of the time the particles reside in the flow channel.

Preferably, the fluid channel is sized so that for efficient separation, particles are displaced 10 - 30  $\mu$ m in the flow channel. Thereby, the force has only to be exposed to a particle over a very short period of time and thus, continuous separation of particles may be performed.

In order to collect the particles, which are deflected in the flow channel, the micro flow system further comprises second outlet means for discharging particles having been deflected in the flow channel.

The micro flow system may comprise second inlet means for entering a first guiding buffer into the flow channel together with the fluid containing particles. When the flow is laminar, the two fluids flow through the flow channel in parallel abutting each other along a small area extending along a longitudinal axis of the flow channel. Particles in the fluid containing particles may then be deflected into the guiding buffer fluid when the two fluids pass the field generated across the flow channel. Furthermore, two (or even more) outlets may be provided at the down stream end of the flow channel for discharging the guiding buffer now containing separated particles and

fluid substantially without particles susceptible to the field generated across the flow channel, correspondingly.

5 The micro flow system may further comprise third inlet means for entering a second guiding buffer for improved control of the path of particle flow through the flow channel. By adjustment of the flow velocities of the guiding buffers and the fluid containing particles, the flow within the flow channel of fluid containing particles may be controlled to flow within a cylinder with a longitudinal axis extending substantially parallel to a longitudinal axis of the flow channel and further the position within the flow channel and the diameter of the flow cylinder may be  
10 controlled by corresponding adjustments of the volumetric ratio between the flow rate of the fluid containing particles and the flow rate of the guiding buffers.

Preferably, the channel depth is small enough, e.g. below 50  $\mu\text{m}$ , to allow observation of the particles flowing through the channel by a microscope. In an important embodiment of the  
15 present invention the micro flow system comprises a cover, e.g. a transparent or translucent cover, for covering the flow channel. When the cover is transparent or translucent, it will be possible to observe event in the flow channel, e.g. passage of a stained or coloured particle or cell.

The member with the flow channel may be produced from any suitable material, such as silicon,  
20 polymers, such as Plexiglas, Teflon, etc., glass, ceramics, metals, such as copper, alumina, stainless steel, etc., etc.

The channel may provided in the member by any suitable manufacturing process, such as  
25 machining, etching, etc.

In a preferred embodiment of the invention, the member is a silicon chip manufactured utilising photolithography and the channel is etched into the silicon chip.

30 The field may be a magnetic field, a electric field, a gravity field, etc., and any combination of such fields. Thus, the field is, in the case where the field is a gravitational field, naturally occurring, and, thus, no field generating means need to be included in the micro flow system, however the flow of the fluids should be arranged as outlined in Fig. 2(b).

35 A magnetic field may be generated by permanent magnets, such as rare earth magnets, such as samarium-germanium magnets, a mixture of ferromagnetic powder and epoxy, etc., etc., electromagnets, e.g., in silicon integrated electromagnets, etc. The magnets are preferably positioned adjacent to the flow channel so that the magnetic field is substantially perpendicular to a longitudinal axis of the flow channel.

In a preferred embodiment of the invention, the magnets are positioned in and glued to rectangular slots that are etched into a silicon chip.

5 The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may be magnetically with stained to facilitate separation in a magnetic field.

In the case where particles have to be detected in a flow channel by optical means, such particles are preferably stained with a chromophoric reagent, e.g. a fluorescent probe.

10

An electric field may be generated by electrodes, such as metal electrodes, such as gold electrodes, etc. The electrode may be position inside the flow channel, e.g. to introduce electrophoretic forces, e.g. for separation of charged molecules in the fluid, or outside the flow channel e.g. to introduce dielectrophoretic forces, e.g. for separation of particles contained in the  
15 flow. Preferably, the electrodes are positioned in such a way that the electric field is substantially perpendicular to a longitudinal axis of the flow channel.

The field generated across the flow channel may be utilised for immobilisation of particles whereby particles may be held in substantially fixed positions within the flow channel for a  
20 specific period allowing chemical reactions with the particles to take place and/or kinetic measurements on the particles to be performed and/or to bring the particles into contact with different chemical substances, e.g. as outlined in Fig. 13. In this case the particles may undergo a washing step before release (removal of the field).

25 As described above, in an interesting embodiment of the present invention the field is a gravitational field or where the separation is performed using gravitational means.

In an further interesting embodiment, the micro flow system according to the invention involves facilities for performing pre-treatment and/or post-treatment of the fluid comprising the  
30 particles. These possibilities are outlined in Figs. 4(f), 8 and 10. As an example, the particles may be treated with a reagent before entering the flow channel, e.g. undergo magnetic or chromophoric staining. Post-treatment may comprise means for collecting or concentrating the deflected particles or means for contacting the deflected particles with one or more reagent(s).

35 By one possible combination of the pre-treatment and the post-treatment facilities, cells may undergo magnetic staining before entering the flow channel, and after separation the staining may be removed by treatment of the stained cells with a suitable reagent.

40 The positions in relation to the flow channel of the field generating means may be adjustable for adjustment of the strength of the field across the flow channel.

It is an important advantage of the present invention that a micro flow system is provided that operates continuously with no requirement for operator intervention.

5 It is another advantage of the present invention that separation is performed in one step.

It is still another advantage of the present invention that the particles are separated in a continuous flow without substantially interfering with the flow itself and that separated particles may be collected at corresponding separated outlets of the flow channel without having  
10 to interrupt the flow in the flow channel.

It is yet another advantage of the present invention that the micro flow system is easily integrated into other continuous flow systems, such as flow cytometers, flow injection analysis systems, etc.

15

It is a further advantage of the present invention that particles may be separated into a plurality of groups of particles, e.g. different subpopulations of cells, based on different susceptibility to the field generated across the flow channel of the different groups of particles. This may be obtained by using a multiple outlet micro flow system as outlined in Fig. 4(c).

20

It is a still further advantage of the present invention that the micro flow system allows observation of particles in the flow channel using a microscope.

25

It is yet another advantage of the invention that a closed system is provided allowing biohazardous samples, such as samples containing pathogens, to be entered into the system without contaminating the laboratory environment and without causing hazard for operators working with pathogen biomaterials.

30

It is a still further advantage of the invention that a system with a low shear stress in the flow is provided allowing a gentle treatment of biological samples, e.g. fragile living cells.

35

According to another aspect of the invention, a micro flow system for analysing components of a fluid is provided that comprises a member having a flow channel defined therein for laminar flow of a fluid, first inlet means for entering particles into the flow channel, first outlet means for discharging fluid from the flow channel and a plurality of areas located in the flow channel and comprising immobilised reagents so that the fluid is analysed for a plurality of components in one operation.

40

The micro flow system of the previous section may further comprise field generating means positioned proximate to at least some of the areas adapted to comprise immobilised reagents,

each field generating means generating a field proximate to the corresponding area whereby reagents entering the flow channel and being susceptible to the field generated at the area are attracted to and immobilised at the area or are rejected from the area.

5 According to an important aspect of the invention, a new system for immunomagnetic cell separation and manipulation is provided that utilises a silicon based micro fabricated flow chip. The system combines the advantage of flow cytometry and immunomagnetic separation technique. The flow chip will be a key component of a portable micro system for cell sorting and analysis. The flow chip is designed for rapid immunomagnetic cell separation without any  
10 pressure drop. Its simple and cheap fabrication and versatile sorting and detection properties offers an alternative to conventional cell separation systems.

It is an advantage of the invention that a micro flow system is provided that is cheap, easy to operate, versatile, simple and portable and that offers the possibility of automation.

15

A miniaturised flow channel system is provided that utilises the advantageous fluid behaviour in micro systems. The invented system operates continuously. Instead of holding back the magnetisable particles in the separation unit, the particles are deflected into the direction of the magnetic field while passing it continuously. By splitting the fluid flow into two or more outlets,  
20 the deflection of the particles can be used for separation of particles into different sets of particles, each of which leaves the flow channel through a specific outlet.

The continuous separation system (CSS) allows efficient enrichment as well as depletion of labelled sample contents of interest. The CSS is designed to fit under a microscope allowing  
25 parallel detection of the optical properties of the sample and the control of separation of particles.

An advantage of the geometry of the invented separation flow channel is that a magnetised or electrically charged particle has to be moved only over a distance of 10 - 30  $\mu\text{m}$  to be separated from the fluid containing particles.

30

Furthermore the invention enables isolation of multiple cell or particle subpopulations from a single sample at the same time. The magnitude and direction of the force  $F$  on a magnetisable particle, e.g. a magnetically labelled cell, is dependent on the magnitude of the magnetic field and the number of magnetic moments inducible on a labelled cell.

35

$$F = N \cdot S \cdot \mu_B \cdot \text{grad } B$$

where  $S$  is the number of Bohr magnetons ( $\mu_B$ ) per particle and  $N$  is the number of particles per cell.

40

Beads with small S are moving a less distance in lateral direction in relation to the flow through the flow channel than beads with a higher S value. This can be used to separate subpopulation of cells labelled with different magnetisable beads: By splitting the flow channel in various outlet channels cells can be distinguish and separated due to their individual F values.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

10

Fig. 1 illustrates the operation of particle separation according to the present invention.

Fig. 2 shows a cross-sectional view of a separation flow channel according to the present invention. (a) shows the main embodiment and (b) shows a cross-sectional view of a separation flow channel for gravitational separation,

15

Fig. 3 shows a flow diagram of a magnetic particle separation apparatus according to the present invention,

20

Fig. 4 shows flow diagrams of various embodiments of the present invention. (a)-(d) show embodiments with various numbers of inlets and outlets, and (e) shows an embodiment with an enlarged separation chamber, and (f) shows an embodiment with an enlarged chamber for collecting separated particles.

25

Fig. 5 shows a flow diagram of two flow channels coupled in parallel (a) and in sequence (b) and (c).

Fig. 6 shows a micro flow system with electrodes as field generating means.

30

Fig. 7 shows a flow channel having an array of electrodes.

Fig. 8 shows a flow diagram for optical detection and hydrodynamic separation using a blocking valve.

35

Fig. 9 shows a flow diagram for optical detection and hydrodynamic separation using a pump.

40

Fig. 10 illustrates the principle of introducing a pre-treatment facility in the member comprising the micro flow system, here further combined with a post-treatment facility or a hydrodynamic separation facility.

Fig. 11 shows a flow channel for magnetophoresis.

Fig. 12 shows diagrams from the magnetic separation described in Example 3.

5

Fig. 13 illustrates entrapment of magnetic particles in a flow channel.

Fig. 14 illustrates the preparation of a micro flow system as described in the Examples.

## 10 DETAILED DESCRIPTION OF THE DRAWINGS

According to a preferred embodiment of the invention, magnetically stained particles, e.g. cells labelled immunologically with magnetic particles, such as antibody-coupled magnetic beads, are separated from non-magnetic particles, i.e. non-labelled cells, by exposing the particles to a  
15 magnetic field generated with a permanent or an electromagnet. Positive or negative selection methods may be employed. By positive cell separation, cells of a specific cell type are separated and isolated from a heterogeneous mixture of cells.

Fig. 1 illustrates the principle of the separation method according to the invention. A micro flow  
20 system 1 is shown having three inlet and two outlet ports. The fluid 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the micro flow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, respectively. A separation magnet 8 is located adjacent to the flow channel 5 and generates a magnetic field  
25 across the flow channel 5. When the fluid 9 containing particles passes the magnetic field, magnetically stained 12 (particles) are drawn into the guiding buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the outlet 6 while non-labelled cells 13 which are not influenced by the magnetic force remain in the fluid 9 leaving the flow channel 5 through the waste drain 7.

30

Due to the small channel dimensions, the flow is laminar with negligible influence of inertia forces. Mixing of the sample stream and the guiding buffers is not detectable since the contact area is small and the contact time is reduced to a subsecond range. The thickness of the sample stream can be precisely adjusted by variation of the flow rate of the two guiding buffers. This  
35 enables the adjustment and optimisation of the magnetic micro flow system for various cell types and sizes.

The magnetic field in the micro flow channel operates as an extremely sensitive filter for magnetic particles, e.g. cells. Cells labelled with superparamagnetic beads (e.g. MACS, Dynal)  
40 are magnetised and attracted by the magnetic field whereby the flow of magnetised particles is

deflected into the separation drain. The short residence time of the fluids in the flow channel and the low Reynolds numbers of the flow in the flow channel minimise the influence of gravity compared to the influence of the magnetic force.

5 Fig. 2 shows a cross-sectional view of two variants of the micro flow system 1 manufactured utilising semiconductor technology. The micro flow system may be manufactured in any suitable material such as polymers, glass, semiconductors, such as silicium, germanium, gallium arsenate, etc., etc.

10 The first micro flow system (a) shown is a 3-layer sandwich. The central layer 14 is a silicon wafer having a flow channel 5 etched into it. The silicon wafer 14 is covered with a transparent plate 16, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel 5 may be observed through the glass plate 16, e.g. utilising a microscope 18 (detection means). The fluid inlet 2 and outlet 7 are connected to tubings 20, 22, e.g. fused silica capillary  
15 or teflon tubings, for entering fluids into or discharging fluids from the flow channel 5. Buffer inlets 3 and 4 and the outlet 6 for the separated particles are not shown. The bottom plate 24, e.g. made of plastic, facilitates mounting of the tubings 20, 22.

A modified version (b) of the micro channel system separation was designed to sort particles due  
20 to their density and/or diffusion constant. For this the system was changed having inlets and outlets above and below the micro channels, respectively, as illustrated in Fig. 2(b). This embodiment of a micro flow system 1 has an inlet port 2 and an outlet port 7 located above the micro channel 5 and an buffer inlet port 3 and a sample outlet port 6 located below the micro channel 5. The fluid containing particles enters the separation flow channel through inlet port 2,  
25 and a guiding buffer enters the separation flow channel 5 through inlet port 3. In this way two laminated layers oriented in the x-y axis are created and these are continuously guided through the separation flow channel 5 of the micro flow system 1. Particles pass over from the particle containing layer into the acceptor buffer layer by sedimentation. When the fluid 9 which contains the particles passes the flow channel 5, particles with certain density properties are  
30 drawn into the acceptor buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the outlet port 6 while particles which are not that mobile (susceptible to the gravitational field) remain in the fluid 9 leaving the flow channel 5 through the waste drain 7. The radial displacement of a specific molecule in the sample is given by its density and diffusional constant and the contact time of the particle containing liquid layer with the acceptor  
35 buffer layer. The contact time is defined by the total flow rate of the liquids passing through the micro systems 1 and the length of the micro channel 5. The sample is introduced from the upper inlet into the separation channel. A buffer containing solution which is used as an acceptor liquid for the separated sample is introduced from the lower inlet into the separation channel. Thus two laminated flow layers orientated in the horizontal axis are created (in contrast to this,  
40 flow channels for optical cytometry or magnetic sorting have all inlets and outlets on the same

plane, thus, vertical layers are created). While the sample passing the separation channel in the longitudinal direction particles are sedimenting into the acceptor buffer due to their density or diffuse into the acceptor buffer due to their diffusion constant. The system can be adjusted that a desirable or appropriate specimen can be withdrawn and separated from the sample flow stream due to their density or diffusional properties. The system can be adjusted by the volumetric flow rates of the acceptor buffer and particle containing flow stream.

Characteristic features of an exemplary embodiment of a micro flow system according to the invention, e.g. as shown in Figs. 1 and 2, is shown in Table 1.

Table 1 Characteristics, micro flow system

---

Manufacturing method Material: Silicium Oxide, SiO<sub>2</sub>

Photo-lithography

Wet-chemical etching

Flow Channel

Cross sectional area 0.1 - 0.55 mm width x 0.04 - 0.2 mm depth

Length 1.0 - 200 mm

total flow rate [ $\mu$ l/min] 1 - 200

flow velocity [mm/min] 15 - 180

Reynolds number 0.1 - 20

separation time 0.1 sec - 6.0 sec [2  $\mu$ l/min]

Magnet

Permanent Magnet

Rare Earth Samarium-Germanium 0.5 x 0.5 x 0.2 mm

Electromagnet

Holding Magnet 25 mm 12 V D.C. RS

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Fig. 3 shows a micro flow apparatus including a micro flow system 1 as shown in Figs. 1 and 2. The micro flow system 1 has two inlets 26, 28 and two outlets 30, 32, two syringe pumps 34, 36, two 3-way control valves 38, 40, and capillary tubings 20, 21. The capillary tubings 20, 21 are used for interconnecting the two syringe pumps 34, 36 with the inlets 26, 28 of the micro flow system 1.

Conventional syringe pumps which are controlled to generate a predetermined volumetric flow rate have been utilised for generating a continuous flow from the buffer reservoir 44 to the inlet tube 26 and a continuous flow from the sample reservoir 42 to the inlet tube 28.

Fig. 4 illustrates various micro flow systems 50, 52, 54, 56, 58, and 59 having flow channels of different geometry. Micro flow systems with two or three inlet ports and two, three or five outlet ports, respectively, are shown with (a)-(d). Micro flow systems with a separation channel with an area having a magnet positioned along a longitudinal axis and an area where the width of the separation flow channel is enlarged are shown with (e). According to the behaviour of liquids in a flow channel, the size cross-sectional area occupied by the sample flow stream is proportional to the width of the micro chamber. According to this the distance between two hypothetical particles A and B rises proportional to the width of the micro chamber. A bigger distance between particles which are to be separated could yield to a higher selectivity of the mechanical separation. (f) show a micro flow system where the width of the outlet channel 6 is enlarged to chamber where the sorted particles are collected for further processing or analysis, e.g. detection, staining, destaining or cultivation.

The magnets may be positioned in rectangular slots that are etched into the silicon wafer 1. The slots are located adjacent to the separation flow channel 5. As shown in Fig. 1, a permanent magnet 8 or a electromagnet 8 can be received by slots in the micro flow system 1. The slots are, e.g., 0.5 mm wide, 0.5 mm long and 0.2 mm deep. For generation of a magnetic field, a solid magnetic block, i.e. rare earth magnet can be glued into the slot. Alternatively, a mixture of ferromagnetic powder and epoxy can be injected into the slots to produce a high magnetic field gradient.

The strength of the magnetic field inside the micro flow system 1 can be adjusted. If an electromagnet is used for generation of the magnetic field, the magnitude of the field may be varied by varying the amplitude of the voltage input to the electromagnet. If a permanent magnet generated the magnetic field, the magnitude of the field may be varied by varying the distance between the magnet and the flow channel of the micro flow system 1.

The diameter or thickness of the flow layers in the micro flow system 1 is adjustable by changing the flow ratio of the sample stream and control buffer stream. Microscopic observation enables a visual control of the flow inside the micro flow system 1. A thickness of the sample stream layer less than 1  $\mu\text{m}$  may be achieved.

As already mentioned, the net displacement of a particle in the micro flow system 1 depends on the force applied to it in the field. This can be utilised for separation of a first group of particles of various types in a fluid into a plurality of set of particles, each set comprising a specific type of particles. A micro flow system 1 with e.g. five separation outlets may be used to separate a fluid containing particles into five sets of particles, each set comprising particles that are influenced by the field with a force of a specific magnitude, in the following denoted particles with a specific F-value. Particles with a low F-value are only deflected by a small amount by the field and are discharged from the flow channel through a corresponding outlet port. Particle deflection is

increased with increasing F-values whereby such particles are discharged from the flow channel through corresponding other outlets.

Fig. 5(a) shows two flow channels 60, 62 operating in parallel. The fluid containing particles enters the flow channels 60, 62 through inlet ports 64, 66, respectively. The guiding buffer enters the flow channels through the inlet ports 68, 70, respectively. In the flow channels 60, 62, particles are separated from the fluid containing particles by the magnetic fields generated by magnets 72, 74, respectively, whereby particles susceptible to the magnetic field are deflected from the fluid containing particles into the corresponding guiding buffer and flow with the guiding buffer to the sort outlet 86. Fluid containing particles without the deflected particles leave the flow channels 60, 62 through corresponding outlets 88, 90. Thus, the separation speed may be increased by utilisation of a plurality of flow channels coupled in parallel.

Fig. 5(b) shows a micro system where magnetic and/or hydrodynamic and/or gravitational and/or diffusional separation can be combined in sequence. In the examples given, a sample is first separated in a magnetic separation chamber, followed by a hydrodynamic separation due to optical properties of the sample, or two magnetic separations are performed in sequence in order to obtain a highly purified product. Thus, it is possible to analyse and distinguish a sample due to optical and magnetic properties. The principles of hydrodynamic separation using optical means is further illustrated in Fig. 8.

Fig. 6 shows a micro flow system 100 utilising electrodes 102, 104 to generate an electric field across the flow channel 106. The electrodes 102, 104 may introduce dielectrophoretic or electrophoretic forces utilised for particle separation. For electrophoretic separation to take place, gold electrodes may be positioned inside and at the walls of the flow channel 106. By applying a voltage across the electrodes, an electrical field is generated substantially perpendicular to a longitudinal axis of the flow channel. The electrical field cause deflection of charged particles or molecules in the flow channel 106 whereby electrically charged particles can be deflected away from the fluid containing particles flowing in the flow channel and into a guiding buffer also flowing in the flow channel and abutting the fluid containing particles in the flow channel.

Fig. 7 shows a flow channel 110 having a plurality of assay sites, each of which has field generating means that may be individually turned on and turned off. The flow channel 110 shown has rectangular electrodes 112 positioned in small grooves at the bottom of the flow channel 110. A voltage can be applied selectively to each electrode 112. Various probes, receptors, indicators, etc. may be attracted to and be immobilised at selected electrodes by applying a voltage to the selected electrodes while a fluid with the corresponding probes, receptors, indicators, etc. flows in the flow channel 110. In this way, a plurality of assay sites

may be created in the flow channel for simultaneous multi-component analysis of a sample fluid flowing in the flow channel after immobilisation of probes, receptors, indicators, etc.

By modification or coating of the micro channel and electrodes specific chemical and mechanical properties can be achieved. By, e.g., coating of the micro channel with an appropriate matrix, e.g. polyurethane, molecules, reagents, indicators which are attracted by electrophoretic forces to an electrode.

For example, DNA which has an overall negative charge is drawn to the electrode surface by a positive bias. The current on the selected electrode is switched off and the molecule remains on the electrode by absorption. To improve binding forces of the probe, e.g. DNA, electrodes coated with a specific layer or matrix, e.g. a polymer such as urethane or a reactive chemical group, can be used. Thus, an encapsulation or immobilisation of the molecule is achieved.

Pre-preparation of the multiple assay sites may be accomplished as follows: Each electrode is loaded with a specific probe in sequential mode. For this specific electrode in the micro channel a voltage is applied. A specific probe, reagent, indicator containing solution, etc. is guided through the micro channel containing the electrode array and is attracted to the electrodes which are applied to a voltage. Subsequently, the voltage is switched off. The next electrode is applied to a voltage and the species in question is guided through the micro channel. Thus, various grooves containing a specific probe, reagent, or indication can be created. Antibodies, fluorescence molecules, DNA, RNA, proteins dyes are examples. As an alternative to the electric force, a magnetic force applied from an array of electromagnet may be used. For analysis, the system with the multiple array sites can be placed in a optical detector, e.g. under a microscope.

Fig. 8 illustrates an interesting embodiment of the present invention. The apparatus consists of a 2-way valve 17 and a micro channel system having a separation chamber with three inlets and two outlets and a collecting chamber 15. The fluid containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the micro flow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, correspondingly. An optical detector 27 is focused on an area inside flow channel 5. The collecting chamber 15 is used to collect and capture the selected particles for post-analysis. During or after sorting the captured sample can be analysed again, using e.g. a microscope 27. By stopping the liquid flow delivery (stopped-flow) particles are entrapped inside the collecting chamber 15 and can be observed for a desired period of time. A desired liquid, for washing, cultivation or staining of a particle or cell, can be added to the collecting chip (reagent addition).

The cell or particle suspension is pumped, by e.g. syringe infusion pump, through the flow channel. The sample is observed and detected by a microscope 27 equipped with controlling

means 19 for controlling the two-way valve 17. These controlling means may comprise an optical detector, e.g. photomultiplier system (PMT). The objective is focused on the measuring point which is located inside the flow channel 5. The dimension of the measuring point is defined by a pinhole 31 positioned in front of the PMT and the magnification. The micro flow system is placed on a x-y-translational stage allowing to move the micro flow system and to define an appropriate area as a measuring point. For light excitation several light sources can be used, e.g. laser, tungsten lamp, photo diode. For optical detection e.g. a photomultiplier, CCD camera/chip, photo diode can be used. For bundling of the light a fibre optic cable, a photo lens, an objective or a light microscope can be used. Various optical detection methods, e.g. fluorescence, absorbance, can be used.

Particles are physically separated (using hydrodynamic forces) according to functions of multiple simultaneous optical measurements on each particle. The photomultiplier (PMT) signals for each particle is transported to a pulse-height analyser also comprised within the controlling means. A selection circuit provides an activating signal whenever a specific particle exhibits photometric properties within a predetermined range. If the PMT signal for a specific particle exceeds a specific value an actuation pulse is produced. The trigger level and the duration for actuation is selected by the operator.

The sorting apparatus was design to achieve a minimal dilution of the separated sample fraction. Hydrodynamic separation of particles can be performed due to the optical, electrical and other properties of the particle containing sample.

The particles suspended in a fluid medium are pumped through a separation flow chip and the cells are observed at the optical axis of the photo detector, e.g. by a spectrophotometer or microscope, and flow to the separation junction. Unselected particles continue to flow out into the waste outlet 7. If a specific cell has optical properties causing an actuation signal, the valve opens causing the liquids to flow to the sort outlet and has been captured inside the collecting chamber 15. The actuation time or pulse the sort valve is switched on is made longer than necessary to ensure that the desired cell has been transported into the sorting channel.

A blocking valve, e.g. piezoelectric drop-on-demand ink-jet printing valve, is attached to the sort outlet channel allowing to block or open the stream passing through the sort outlet. The flow restriction in the waste outlet channel is much higher as the flow restriction of the sort outlet channel. This can be achieved by attaching a flow restrictor to the waste outlet channel. Thus if the sort outlet channel is not blocked the particle stream is deflected to the sort outlet channel. For analysis operation the sorting channel is continuously blocked by the sort valve. Thus, in this mode all the particles passing through the detection area which is defined by the slit in front of the photomultiplier are drained into the waste container. The trigger level and the duration for actuation is selected by the operator.

An example for an optical and mechanical arrangement of the sorter based on fluorescence detection is illustrated schematically in Fig. 8. The sample, e.g. particles suspension, is guided and centred in a horizontal laminar sheath flow orthogonal to the optical axis. The particle stream is illuminated with the light of a mercury arc lamp passing excitation filters for e.g. fluorescein measurement. A dichroic mirror reflects the excitation light to the sorting chip via a e.g. 20x microscopy objective. The fluorescence light emission is collected by the same objective passing a dichroic mirror. Behind the mirror a slit works as field stop limiting the detection area to a small stripe. Each particle passing the detection field is generating a short photomultiplier signal. The measuring signal is amplified and passed to a peak detector.

Various types of separation flow channels described in Fig. 4 can be used in combination with a blocking valve together with an apparatus which is constructed according to Fig. 3. The system is designed that the flow restriction in the waste outlet channel is bigger than in the sort outlet. Thus all liquids pumped through the separation flow channel are forced into the sort outlet. The sort outlet is connected to a 2-way valve allowing to control the direction of the sample flow. If a cell needed to be separated the valve opens for a short period of time.

The sample is entering the separation flow channel via the central inlet and is guided by two guiding buffers to the interrogation point and sorter junction. The sort outlet is normally blocked by the closed sort valve. Thus all flow stream is guided to the waste outlet. Unselected particles/cells continue to flow out into the waste outlet container. If a specific cell has optical properties causing an actuation signal, the sort valve opens for a short interval of time, causing the fluid to flow out through the sort channel into the collecting chamber. The delay time between detection and actuation of the sort valve is adjustable. The actuation time is adjusted long enough to ensure that a desired cell is guided to the sort outlet. The liquid volume guided to the sort outlet is defined by the time interval the valve is open and by the total flow rate. The actuation speed of the valve used in this device is 1500 MHz which corresponds to a minimal actuation time of 0.6 msec. The sorted particles can be observed, detected in the collecting chamber parallel or after the sorting procedure using e.g. a microscope focused to an desired area inside the collecting chamber.

Because the sort outlet channel only opens when a cell has to be sorted, dilution of the sorted cell fraction is minimal. After a sample has been separated it can be withdrawn from the micro conduit: By flushing the collecting chamber with an appropriate buffer entering the micro conduit via an inlet channel all sorted cells can be collected in sort outlet container.

Fig. 9 illustrate separation method according to the invention. A separation flow channel is used according to Fig. 4 having two inlets and two outlets. The fluid containing particles enters the separation flow channel as describe above. Stepper motor driven syringe pumps are contacted

two outlet port 6 and 7. The stepper motor driven syringe pumps are operating in the reverse mode, meaning sucking the sample and buffer solution via inlet 2, 3, and 4 through the separation flow channel. The cells suspended in a fluid medium are pumped through a separation flow chip. The cells are observed at the optical axis of the spectrophotometer or microscope and flow to the separation junction. Unselected cells continue to flow out into the waste outlet container. If a specific cell has optical properties causing an actuation signal, stepper motor of pump 25 is actuated and stepper motor of pump 23 is stopped causing the liquids to flow to the sort outlet. The actuation time pump 25 is switched on, respectively pump 23 is switched off is made longer than necessary to ensure that the desired cell has been transported into the sorting channel.

Fig. 10 illustrates a micro flow system with integrated steps for automated labelling of the sample with fluorescence or magnetic probes. The systems may be combined with a post-treatment facility for removal of the probes or for any other treatment of the separated particles. The system contains a micro conduit containing separation chamber, channels for addition of liquids, e.g. reagents for cell lysis or staining, incubation and cultivation or storage for further processing. A sample is introduced into the micro conduit, via an inlet and several reagents can be added continuously to the sample and is transported into the incubation chamber. A simple micro conduit structure was constructed for sample pre-treatment. The incubation time between mixing and analysis is given by the volumetric flow rate of the syringe pumps. The technique employs computer-controlled precision drive syringe pumps.

Fig. 11 shows A micro system designed for magnetic separation of macromolecules, i.e. ribonucleic acid, proteins. Magnetic beads are labelled with a fluorescence dye and a probe, specific for i.e. DNA are added to the sample and incubated. The sample containing liquid is introduced via inlet port 2 into the separation chamber 5 and drawn by the magnetic field along the separation channel due to their mobility. After a defined time interval the magnetic field is removed and the fluorescence banding can be observed under a microscope. By running standards of known size it is possible to calibrate the system and to separate DNA due to their size and shape, similar to electrophoresis.

Fig. 13 illustrates that it is possible to entrap particles inside the separation flow channel for a desired period of time using the electromagnet equipped apparatus. In this case the magnetic field is adjusted such that positive magnetic particles are drawn to the surface of the micro channel close to the magnetic poles. Upon removal of the current the particles are redispersed and are rapidly moved to the sorting outlet port. This 2-step sorting procedure could be alternatively used to a continuous sorting procedure. Especially in sorting of extremely rare events where dilution of the sorted cell fraction could be a problem. The figure shows entrapped positive magnetic particles in the process of being withdrawn from a continuous sample flow stream. The magnetic particles are attracted by the magnetic field and withdrawn from the

sample stream by precipitation at the inner wall of the micro channel at the magnetic pole. By removing the dc excitation of the electromagnet the particles have been released into the flow stream again.

## 5 EXAMPLE 1

A micro flow system has been tested utilising it for separation of various magnetisable particles. The test conditions are listed below.

10	Particle concentration	10 <sup>7</sup> particles/ml
	Total flow rate	25 µl/min
	Length flow chip	3.5 mm
	Separation time	2.4 sec
	Desired particle deflection:	10 µm

15

The separation efficiency (enrichment rate)  $E$  and depletion rate  $1/E$  are defined by

$$E = \frac{\frac{\% \text{ positive particles after separation}}{\% \text{ negative particles after separation}}}{\frac{\% \text{ positive particles before separation}}{\% \text{ negative particles before separation}}}$$

For separation of various paramagnetic standard beads of different sizes and paramagnetic field strength, the results are shown in the Table 2.

Table 2 Separation efficiencies

	Paramagnetic Bead	Size $\mu\text{m}$	Separation Efficiency [%] <sup>1</sup>		
			A)	B)	C)
5	<b>Polysciences</b>				
	25 % iron-oxide	1-10	>99	>99	95
	50 % iron-oxide	1-10	>99	>99	96.5
	<b>Paesel + Lorei</b>				
10	Magnetic Affinity	0.5-1.5	>99	>99	97.5
	<b>Boehringer</b>				
	Streptavidin Magnetic	1	90.5	88.7	89.5
	<b>Dynal</b>				
	Magnetic Mass Dyal M-450	1-10	98.0	>99	96.5

<sup>1</sup> total flow rates: A)= 10  $\mu\text{l}/\text{min}$ , B)= 50  $\mu\text{l}/\text{min}$ , C)= 100  $\mu\text{l}/\text{min}$

#### EXAMPLE 2

20 Further, the micro flow system has been tested utilising it for separation of Human T-lymphocytes (JURKAT cells). Magnetically stained and unstained JURKAT cells were used to form a heterogeneous cell sample. For magnetic staining of the cells a CD4-magnetic surface marker from Miltenyi Biotech was used (JURKAT cells were suspended in 1% PBS/BSA to a concentration of  $10^7/\text{ml}$ . Biotin-conjugated CD4 magnetic microbeads were added at 2  $\mu\text{l}$  stock/ $10^7$  cells following manufacturers instruction).

The magnetically stained cells ( $10^7$  cells/ml) flowed through the microchip for 10 min and fluids were collected at two outlets. Three experiments at different flow rates (5, 25, 50  $\mu\text{l}/\text{min}$ ) were performed. The same experiments were performed using magnetically unstained cells.

30 An aliquot of the collected samples were analysed under a microscope and the particles were counted using a Neubauer microscopy chamber. For each experiment 1  $\mu\text{l}$  sample was analysed:

Run	flow rate [μl/min]	cells [%] at Sort outlet
5	negative (unstained cells)	
	5	<0.1
	25	<0.1
	50	<0.1
	Control <sup>1</sup>	
10	5	n.n.
	25	n.n.
	50	n.n.
	Positive (stained cells)	
	5	95.5
15	25	92.8
	50	80.5
	Control <sup>1</sup>	
	5	n.n.
	25	n.n.
20	50	n.n.

<sup>1</sup> using the micro flow system without an integrated magnet

### EXAMPLE 3

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The system for removal of magnetisable particles from a sample consists of two syringe infusion pumps (Harvard Apparatus, Southnatic, Az) that provides constant flow rates of 0.1 to 100 μl/min using a 0.5 ml micro syringe (Hamilton, Bonaduz, Switzerland), a separation flow channel (SFC) of silicon for the removal of the magnetisable particles, and a collecting unit for

30 collecting of the separated sample. Two 3-way microvalves (Lee, Parameter AB, Sweden) were integrated into the apparatus for sterile solution handling. All components were interconnected with fused silica capillaries (340 μm id., Supelco, U.S.A.). The SFC was placed under an inverted microscope (Axiovert 100, Zeiss, Germany) for visualisation of the sorting procedure. All micro channels and tubing have been deactivated by silanisation as described in Blankenstein, G.

35

Scampavia L, Branebjerg J, Larsen UD, Ruzicka J (1996): Flow switch for analyte injection and cell/particle sorting in Analytical Methods and Instrumentation, μTAS '96 conference, 17-22 November 1996, Basel. A FACScan with 488 nm argon laser excitation and collection of forward and side scatter and fluorescence of fluorescein were used (Becton Dickinson, Mountain View, CA) for all experiments. Results were collected and analysed using the FACScan research

40

software (Becton Dickinson).

Results on separation flow channel equipped with a permanent magnet optimised for Dynal beads are shown in Fig. 12. A bead suspension of  $1.5 \times 10^8$  particles/ml containing a mixture of non labelled magnetic Dynal particles (d:  $4.5 \mu\text{m}$ , M-450) and fluorescence calibration beads (d:  $3.2 \mu\text{m}$ , Dako A/S, Glostrup, Denmark) have been separated. The non-magnetic, deflected fraction was collected at the waste outlet in about 1 ml and analysed by flow cytometry (B). To enumerate the positive and negative fractions, two windows were set for the statistic evaluation. Before separation the sample contained 38.3 % fluorescence particles and 55.8 % magnetic particles, respectively (A). After sorting by the described system almost all magnetic particles were found in the sorted fraction collected from the sort outlet (B) and non-magnetic particles were found in the negative fraction (C) collected from the waste outlet, respectively. Under optimised conditions an enrichment rate of 350 was achievable.

#### 15 EXAMPLE 4

The description of the preparation of the micro flow system is supported by Fig. 14.

A separation flow channel was designed to fit into a system realised in a bonded silicon/glass sandwich (Fig. 14). The micro channels are etched in a silicon wafer covered with a boron glass plate (thickness:  $0.2 \text{ mm}$ ), which allows the external observation and detection of the cells suspension inside the micro channels using i.e. a microscope. The separation flow channel was fabricated on a  $4"$ ,  $350 \mu\text{m}$ ,  $\langle 100 \rangle$  silicon wafer. A  $1.5 \mu\text{m}$   $\text{SiO}_2$  was patterned with a mask containing the channel layout. A  $2.6 \mu\text{m}$  photoresist was spun on top of the oxide and patterned with a mask defining intermediate holes. The two step oxide and photoresist mask was used for etching a two level structure with vertical walls by reactive ion etching (RIE) in a  $\text{SF}_6/\text{O}_2$  plasma. The holes were initially etched down to  $22 \mu\text{m}$  and then etched further down together with the channels, which was etched down to depths varying from  $40 \mu\text{m}$  to  $100 \mu\text{m}$ . A  $1.8 \mu\text{m}$   $\text{SiO}_2$  was patterned with a mask for inlets and outlets on the backside. The etching was carried out in KOH at  $80^\circ\text{C}$  and stopped when all the intermediate holes were clearly visible from the backside. Finally, a glass wafer was anodically bonded to the silicon wafer. The micro channels were designed for a volumetric flow rates of  $0.1$  to  $200 \mu\text{l}/\text{min}$  with a mean flow speed of maximal  $100 \text{ (mm/min)}$ .

The separation flow channel is provided with one or two permanent or electromagnets) in three different ways:

- (a) Rare earth Samarium-Cobalt block magnets of  $1 \times 1 \times 0.5 \text{ mm}$  (Goudsmit, Netherlands) were glued with silicon rubber into the opening slot of the separation flow channel.
- (b) Rare earth (Sr) magnetic powder (Tropag, Hamburg, Germany) were mixed with epoxy 1:1 (v/v) and the magnetic paste glued into the opening slot of the separation flow channel yielding to a thick film magnetic layer of  $1.0 \times 1.0 \times 0.5 \text{ mm}$ .

(c) Ferrite steel wool were glued with silicon rubber into the opening slot of the separation flow channel. A high magnetic field gradient can be induced inside the opening slots by applying an external magnetic field. For this an electromagnet (Goudsmit, Netherlands) was used which was placed close to the separation flow channel.

5

## CLAIMS

1. A micro flow system (1) for separating particles (12,13), comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) containing the particles (12,13) in the channel (5), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for discharging fluid from the flow channel (5) and field generating means (8) positioned proximate to the flow channel (5) for generating a field substantially across the flow channel (5) whereby particles (12) entering the flow channel (5) and being susceptible to the field generated across the flow channel (5) are deflected in the flow channel (5).
2. A micro flow system according to claim 1, wherein the Reynolds number for the flow of the fluid through the channel (5) is in the range of 0.01-500, preferably in the range of 0.05-50, in particular in the range of 0.1-25.
3. A micro flow system according to claim 1, wherein the lowest cross-sectional area of the channel (5) is in the range of 0.004-0.11 mm<sup>2</sup>.
4. A micro flow system according to any of the claims 1-3, further comprising second outlet means (6) for discharging particles (12) having been deflected in the flow channel (5).
5. A micro flow system according to any of the claims 1-4, wherein the field generating means comprise a magnet.
6. A micro flow system according to any of the claims 1-4, wherein the field generating means comprise an electrode.
7. A micro flow system according to any of the preceding claims, wherein positions in relation to the flow channel of the field generating means are adjustable for adjustment of the strength of the field across the flow channel.
8. A micro flow system according to any of the preceding claims, further comprising flow speed adjustment means for adjustment of the time the particles reside in the flow channel.
9. A micro flow system according to any of the preceding claims, further comprising a cover (16) for covering the flow channel (5).
10. A micro flow system according to claim 9, wherein the cover is a transparent or translucent cover allowing observation of events in the flow channel.

11. A micro flow system according to any of the preceding claims, further comprising second inlet means (3) for entering a first guiding buffer (10) for reception of deflected particles (12).

12. A micro flow system according to claim 11, further comprising third inlet means (4) for entering a second guiding buffer (11) for control, by the first and second guiding buffers (10,11), of the flow path of the fluid containing particles (12,13) through the flow channel.

13. A micro flow system according to claim 12, wherein the width and the position of the flow of fluid (9) containing particles is controlled by adjustment of the volumetric ratio between the particle flow rate and the flow rate of the guiding buffers (10,11).

14. A micro flow system according to any of the preceding claims, wherein the deflected particles (12) comprise living cells.

15. A micro flow system according to any of the preceding claims, wherein the deflected particles (12) comprise chromosomes, organelles, biomolecules, or proteins.

16. A micro flow system according to any of the preceding claims, wherein the deflected particles (12) have been magnetically stained.

17. A micro flow system according to any of the preceding claims, comprising a plurality of outlets for discharging of particles separated according to their different susceptibility to the field across the flow channel.

18. A micro flow system according to any of the preceding claims, wherein the member comprising the flow channel further comprises pre-treatment and/or post-treatment facilities.

19. A micro flow system according to claim 18, wherein the pre-treatment facilities comprise incubation means for preparing or pre-reacting the fluid (9) comprising the particles (12,13).

20. A micro flow system according to claim 18 or 19, wherein the pre-treatment facilities comprise means for magnetic or chromophoric staining.

21. A micro flow system according to claim 18, wherein the post-treatment facilities comprise means for collecting or concentrating the deflected particles (12).

22. A micro flow system according to claim 18, wherein the post-treatment facilities comprise means for contacting the deflected particles (12) with one or more reagent(s).

23. A micro flow system (1) for separating particles (12,13), comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) containing the particles (12,13) in the channel (5), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for discharging fluid from the flow channel (5), a transparent or translucent cover (16) for covering the flow channel and allowing observation of events in the flow channel, wherein events in the flow channel activate controlling means (19) for controlling the flow through the second outlet means (6), and optionally through the first outlet means (7), said controlling means (19) being connected with optical detection means (27) which is adapted to detect said events.

24. A micro flow system (1) for analysing components of a fluid (9), comprising a member having a flow channel (5) defined therein for laminar flow of the fluid (9), first inlet means (2) for entering particles (12,13) into the flow channel (5), first outlet means (7) for discharging fluid (9) from the flow channel (5) and a plurality of areas (112) located in the flow channel and comprising immobilised reagents so that the fluid is analysed for a plurality of components in one operation.

25. A micro flow system according to claim 24, further comprising field generating means (8) positioned proximate to at least some of the areas adapted to comprise immobilised reagents, each field generating means (8) generating a field proximate to the corresponding area whereby reagents entering the flow channel (5) and being susceptible to the field generated at the area are attracted to and immobilised at the area or are rejected from the area.

26. A micro flow system (1) for separating particles (12,13), comprising a member having a flow channel (5) defined therein adapted for flowing of a fluid (9) and an acceptor buffer (10) in the channel (5), first inlet means (2) for entering an upper fluid (9) comprising the particles (12,13) into the flow channel (5), upper outlet means (7) for discharging fluid from the flow channel (5), second inlet means (3) for entering an lower fluid (10), lower outlet means (6) for discarding fluid comprising particles (12), the flow of within the flow channel being adapted so that at least a part of the particles, due to the higher density relative to the upper fluid (9), are allowed to sediment into the lower fluid (10).

27 A micro flow system according to claim 26, wherein the Reynolds number for the flow through the channel (5) is in the range of 0.01-500, preferably in the range of 0.05-50, in particular in the range of 0.1-25.

28. A micro flow system according to claim 26 or 27, wherein the lowest cross-sectional area of the channel (5) is in the range of 0.004-0.11 mm<sup>2</sup>.

29. A method of separating particles, comprising the steps of

(a) entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, and

- 5 (b) generating a field substantially across the flow channel whereby particles flowing through the flow channel and being susceptible to the field generated across the flow channel are deflected in the flow channel.

- 10 30. A method of analysing components of a fluid, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of sites, each of which comprises immobilised reagents whereby the fluid can be analysed for a plurality of components while it is flowing through the channel.

- 15 31. A method of forming sites comprising immobilised reagents in a flow channel, the method comprising generating a field proximate to the corresponding site while a reagent is flowing proximate to the site, the reagent being susceptible to the field generated at the site whereby the reagent is attracted to and immobilised at the site.

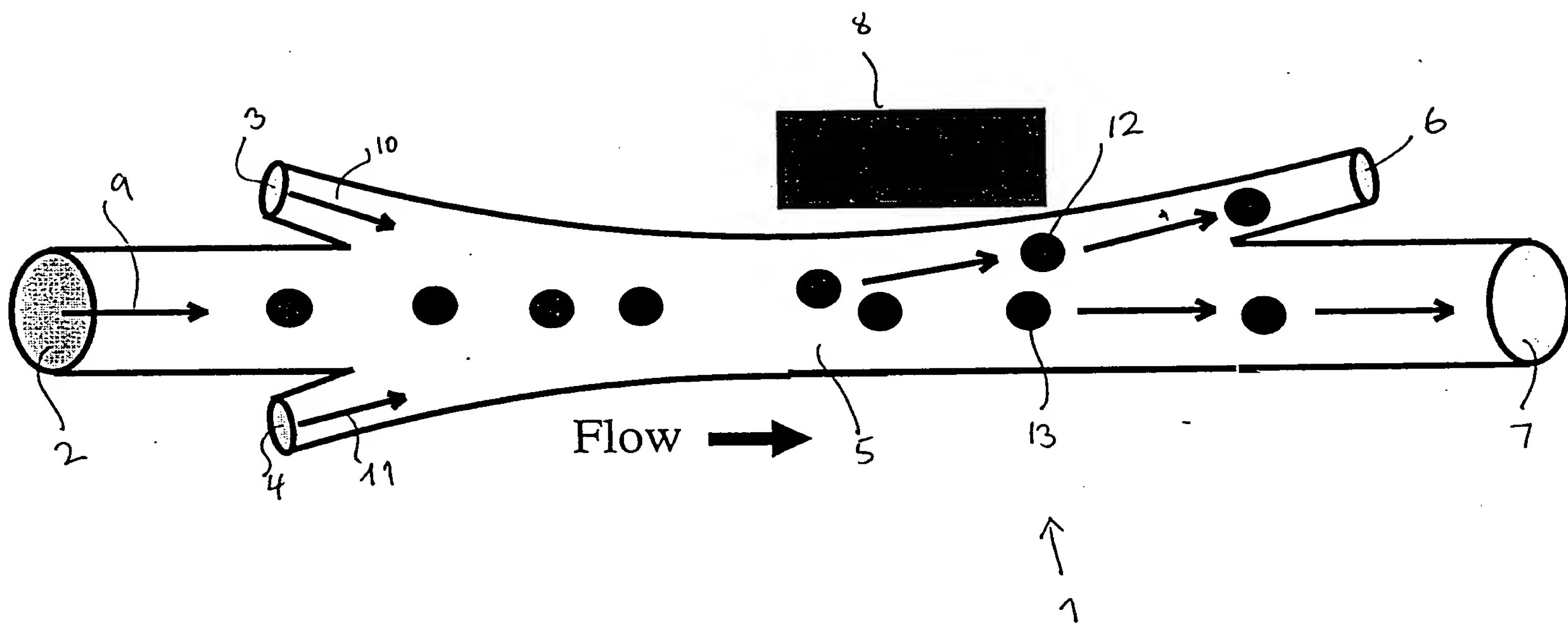
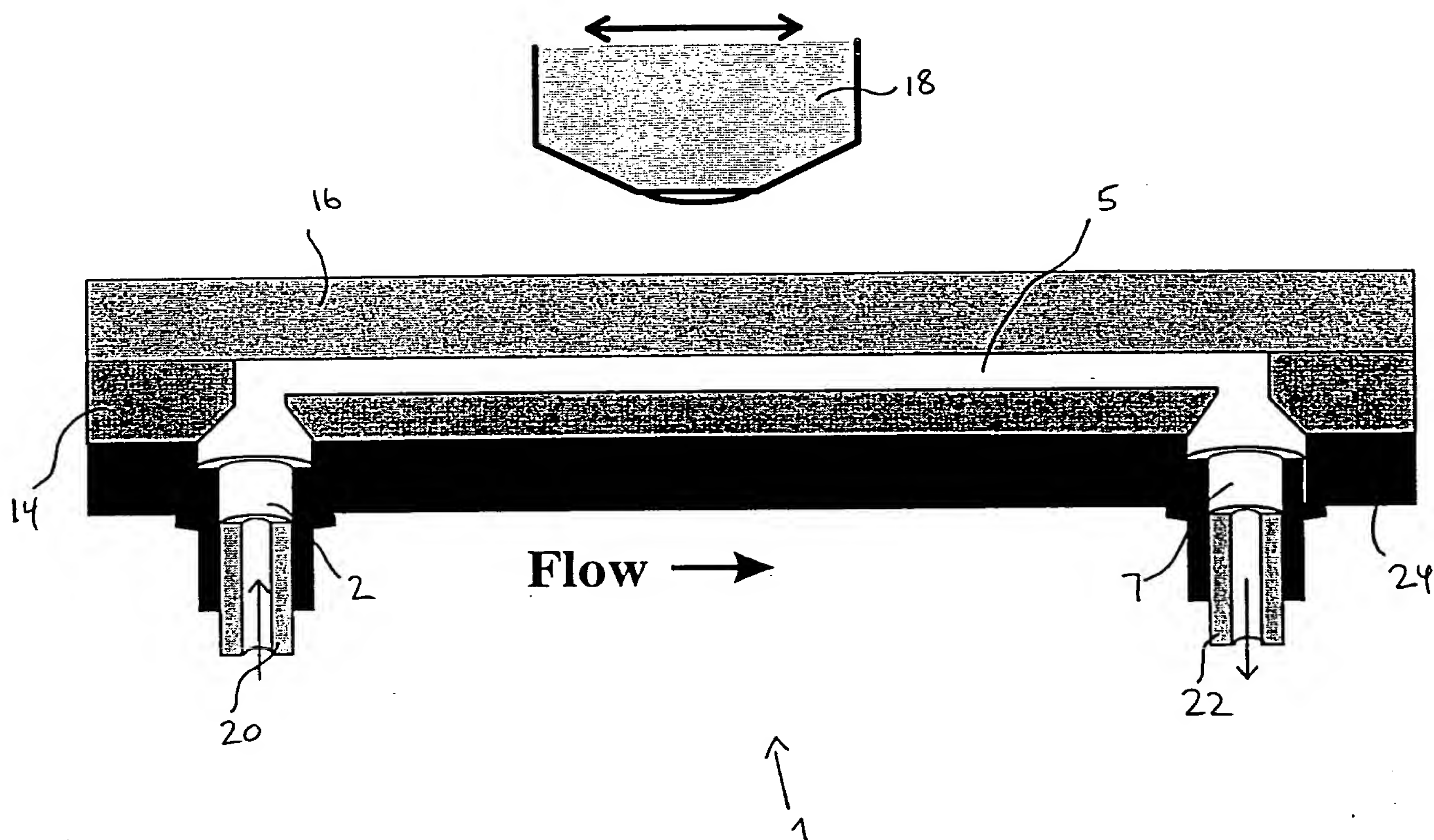


Fig. 1

(a)



b)

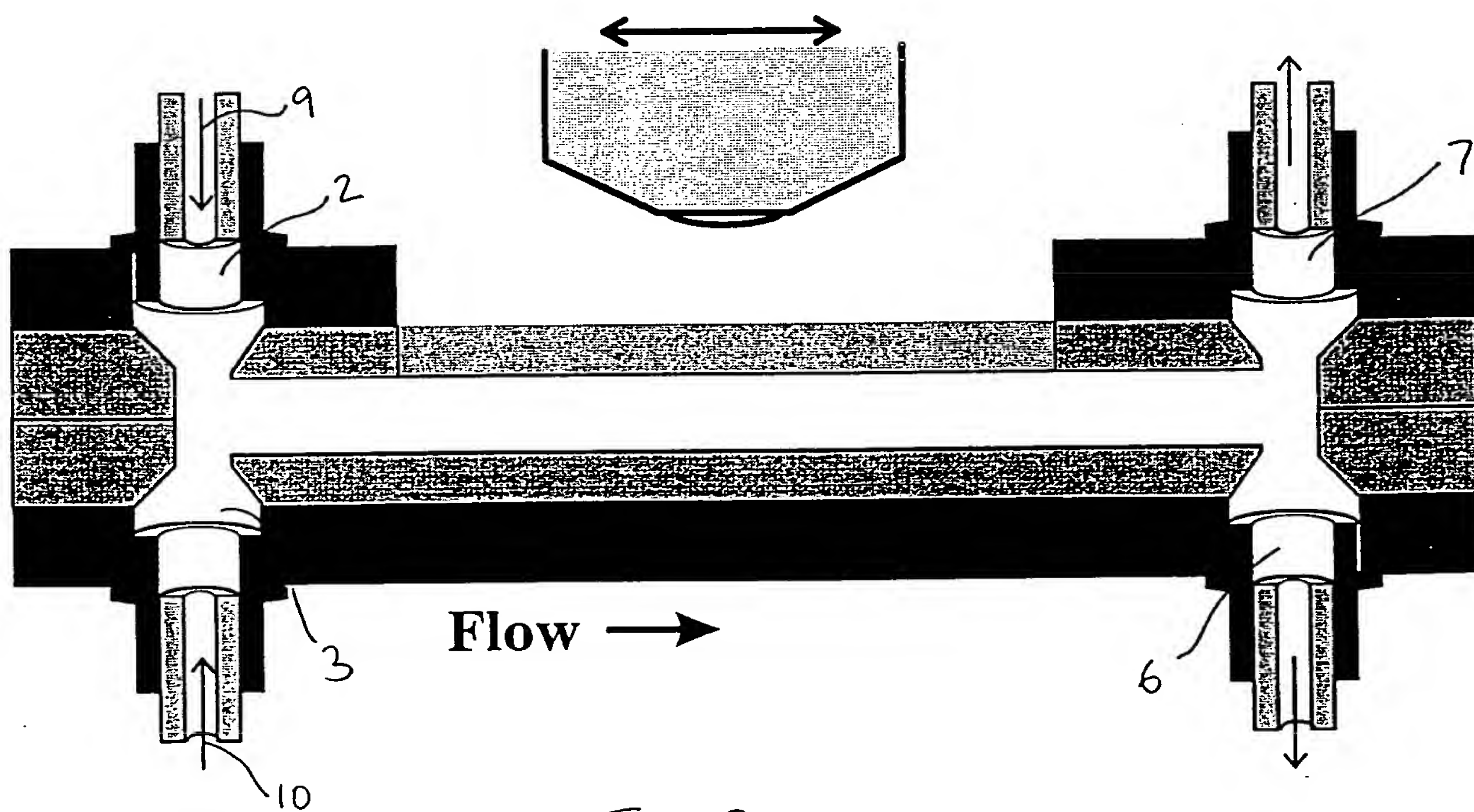
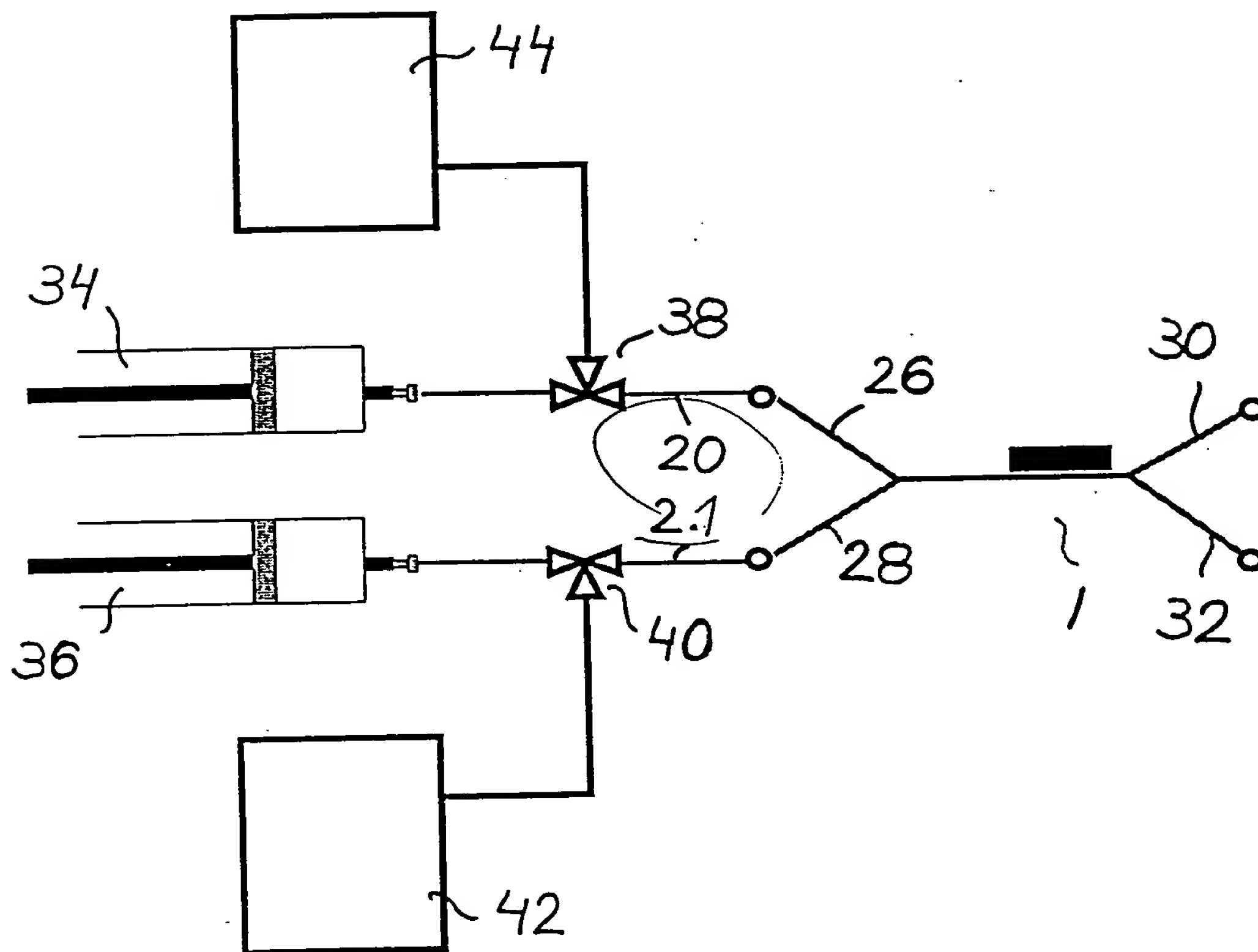


Fig. 2



**Fig. 3**

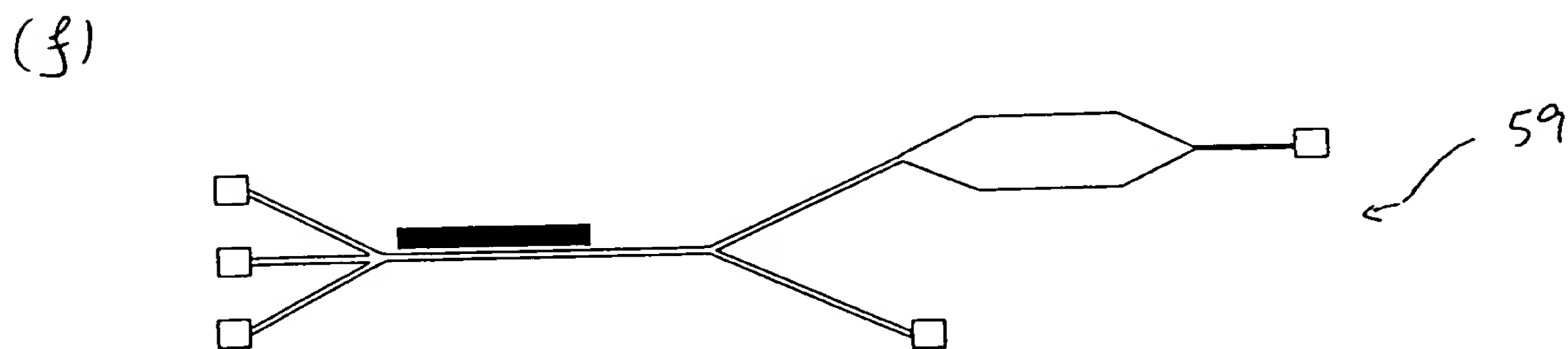
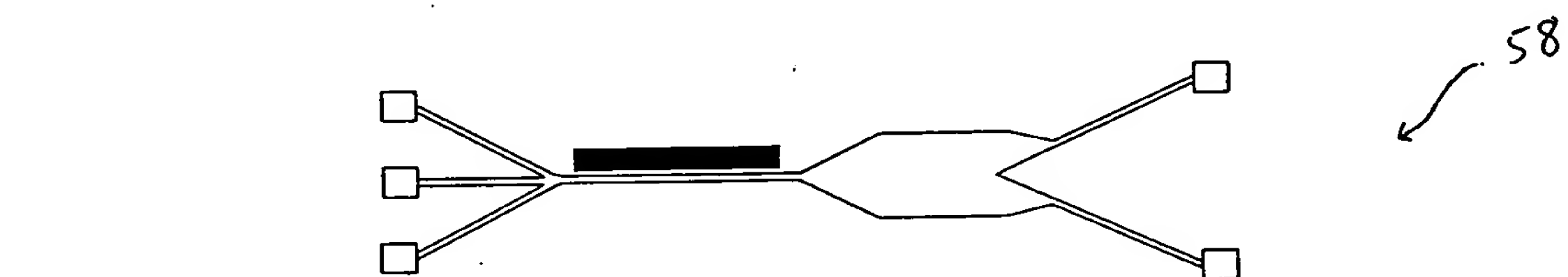
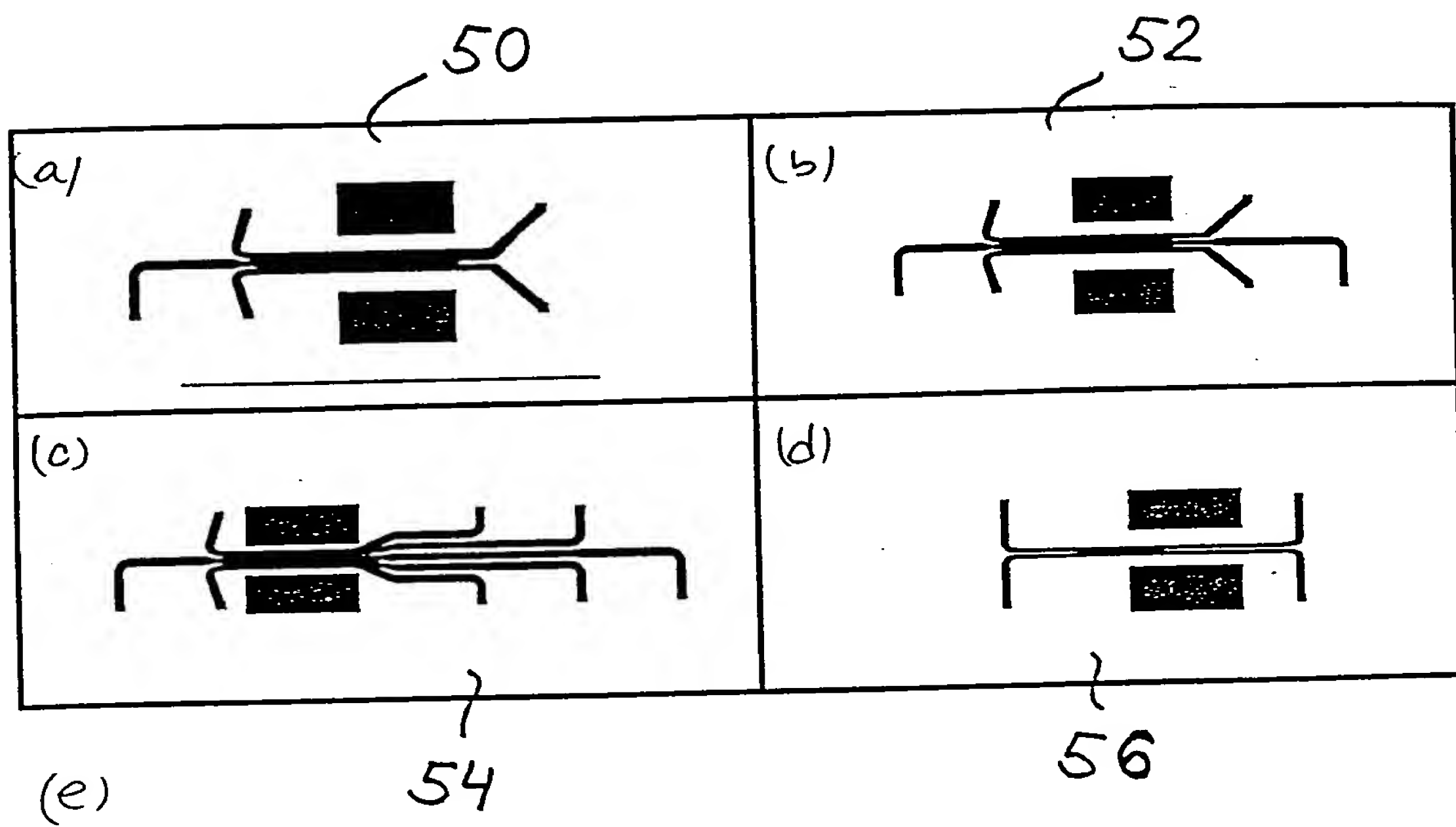
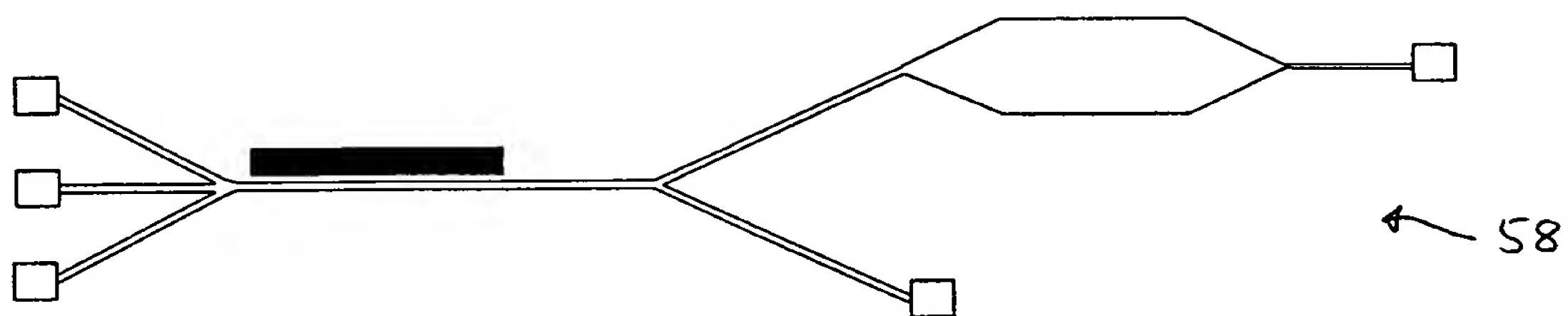


Fig 4.

(e)



(f)

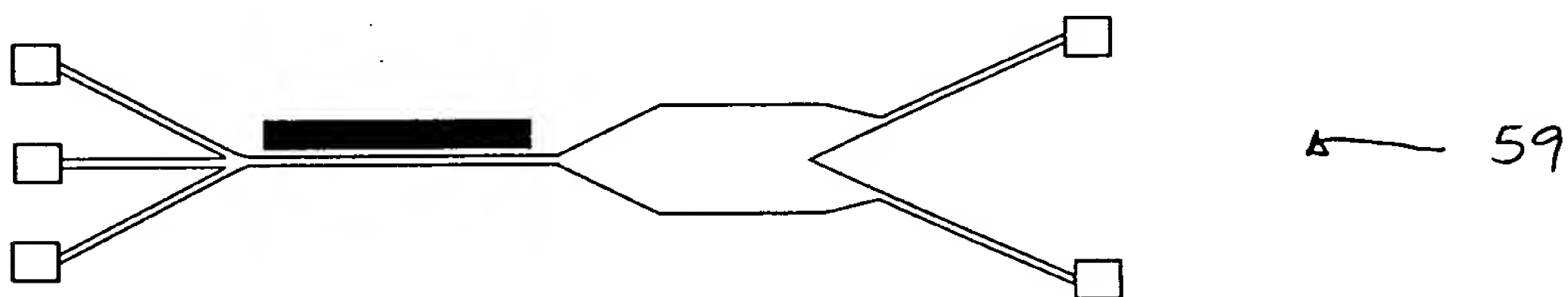
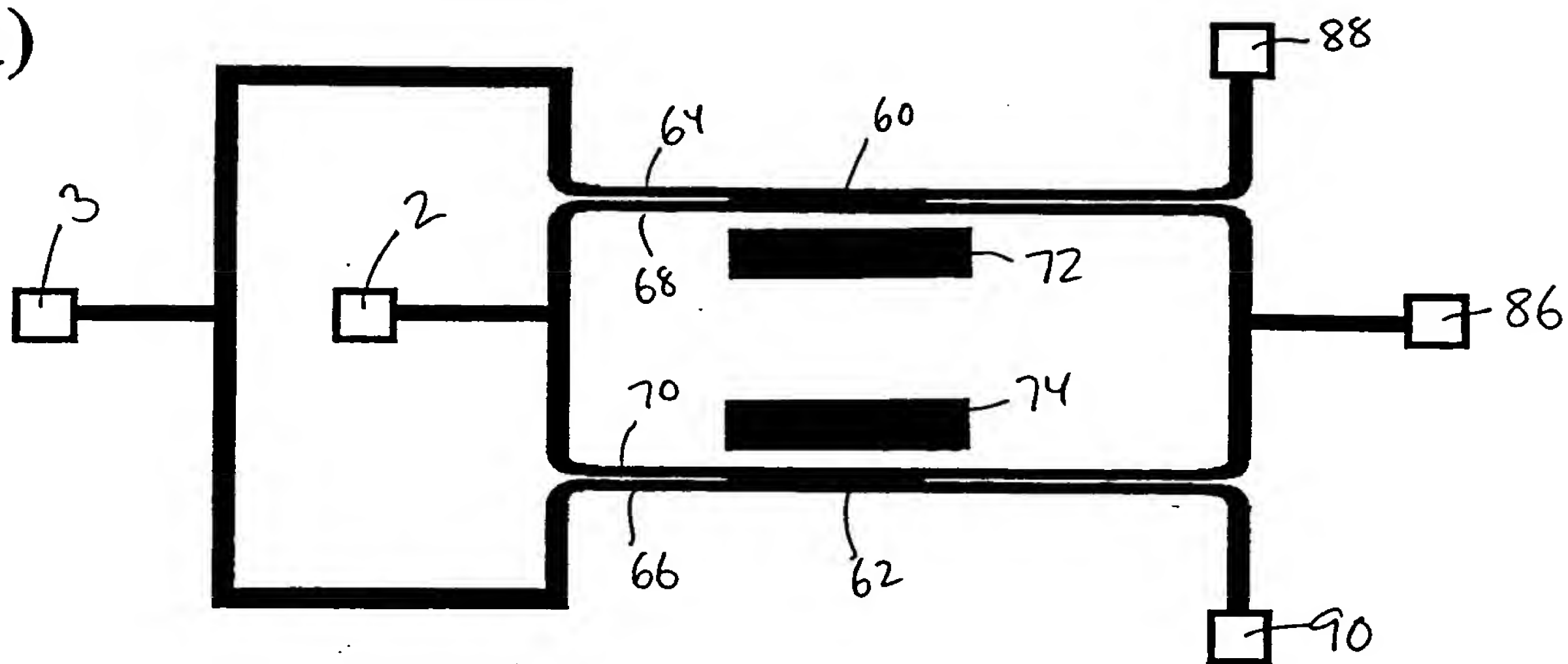
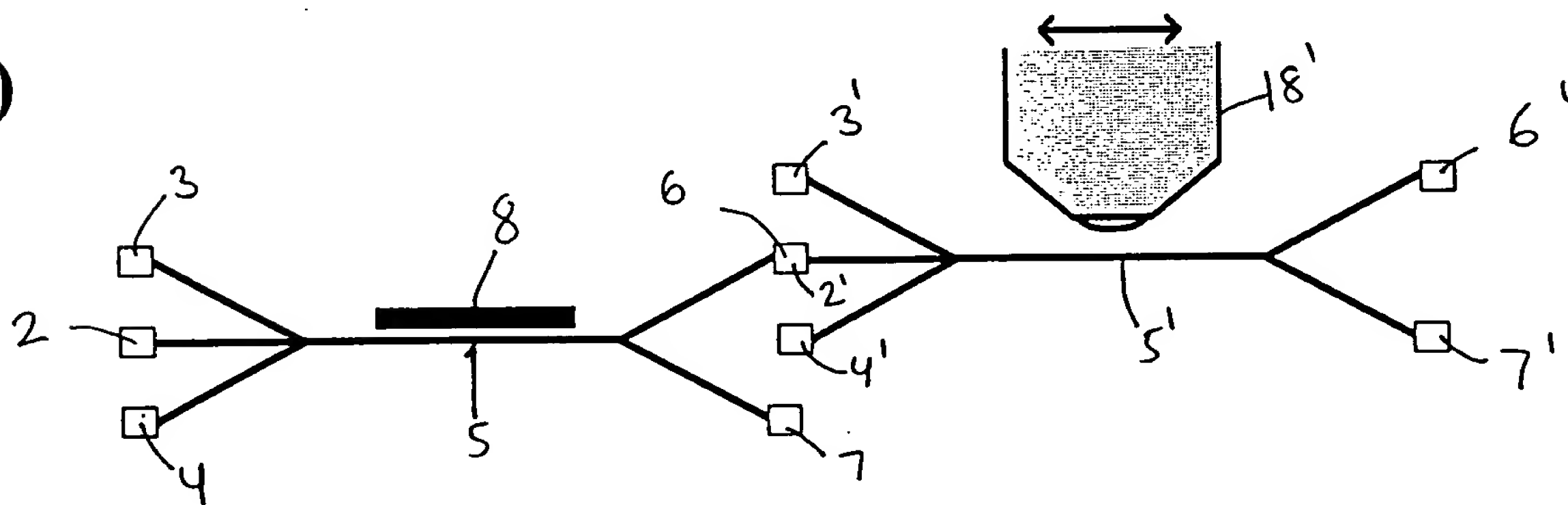


Fig. 4

(a)



(b)



(c)

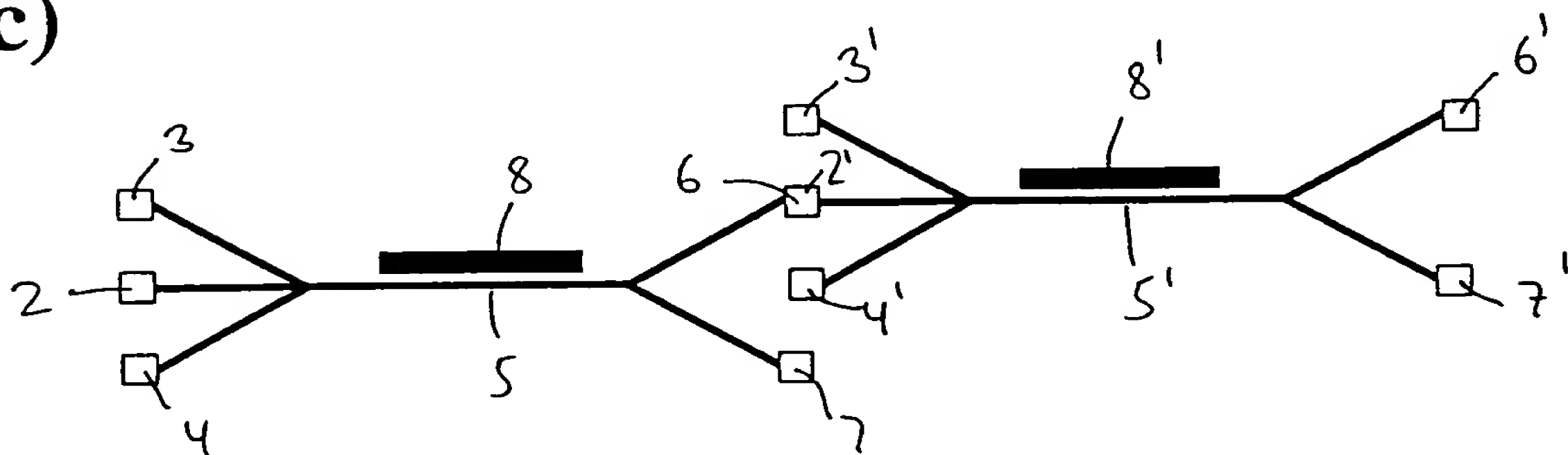
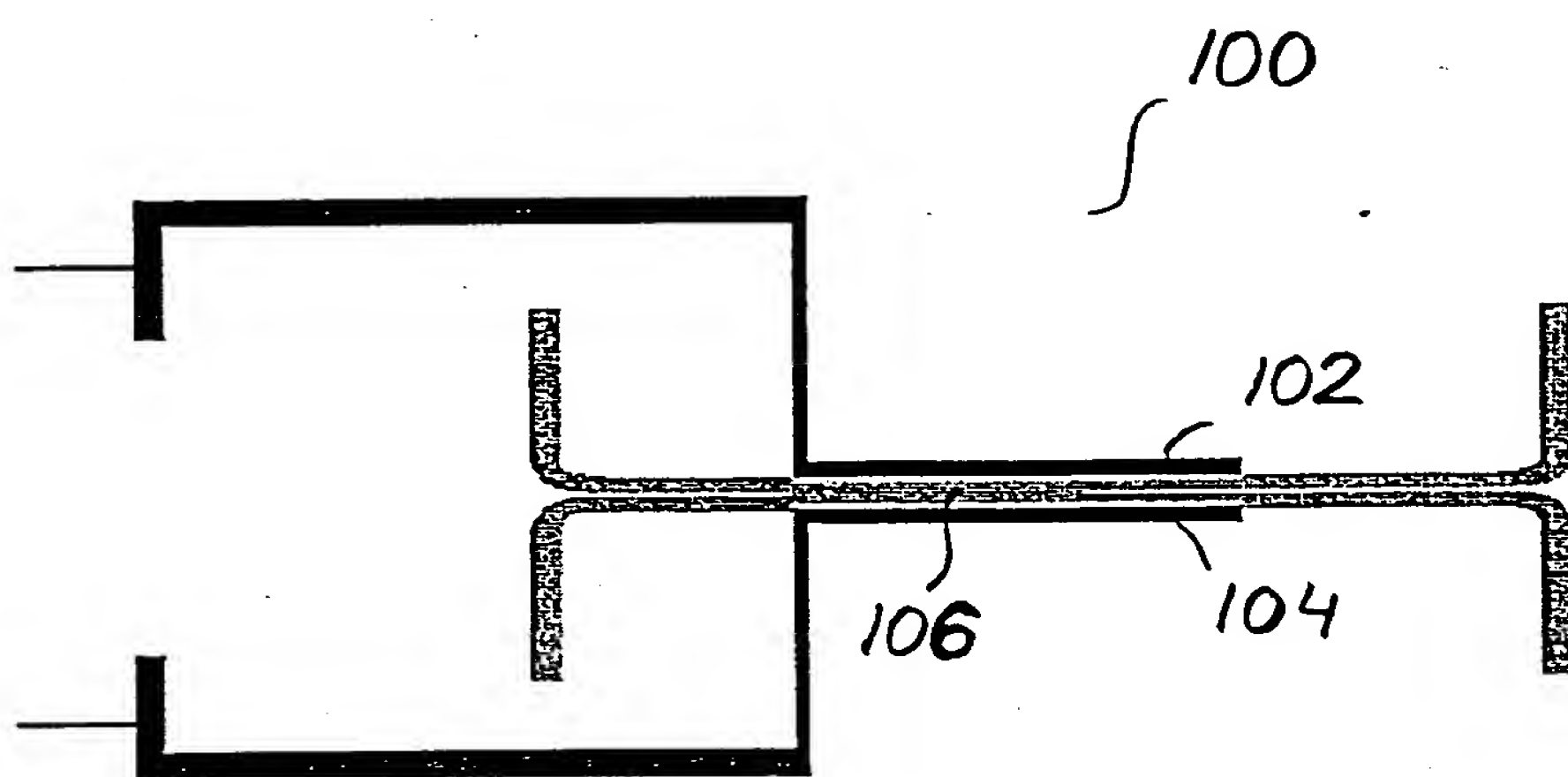


Fig 5



**Fig. 6**

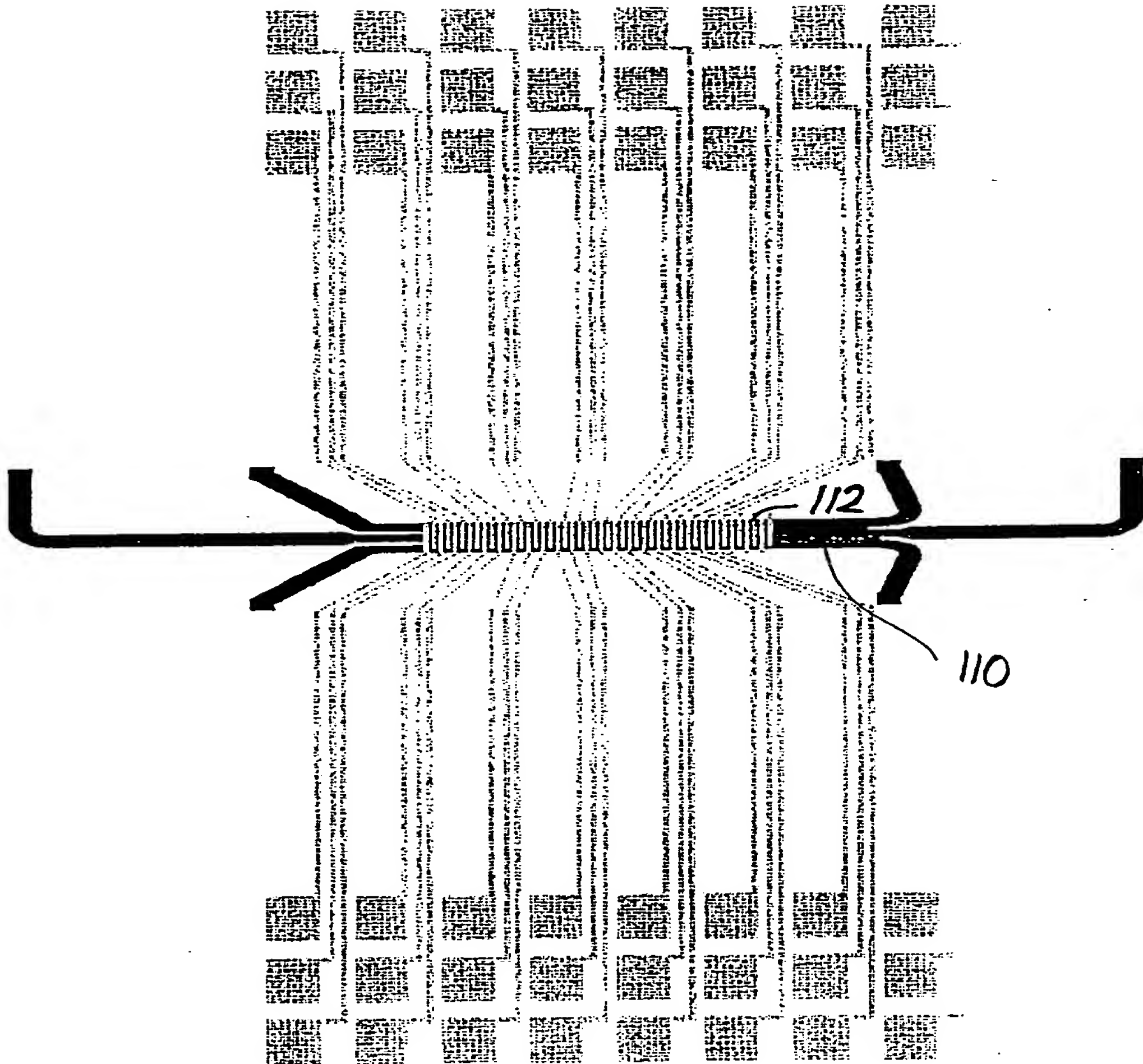


Fig. 7

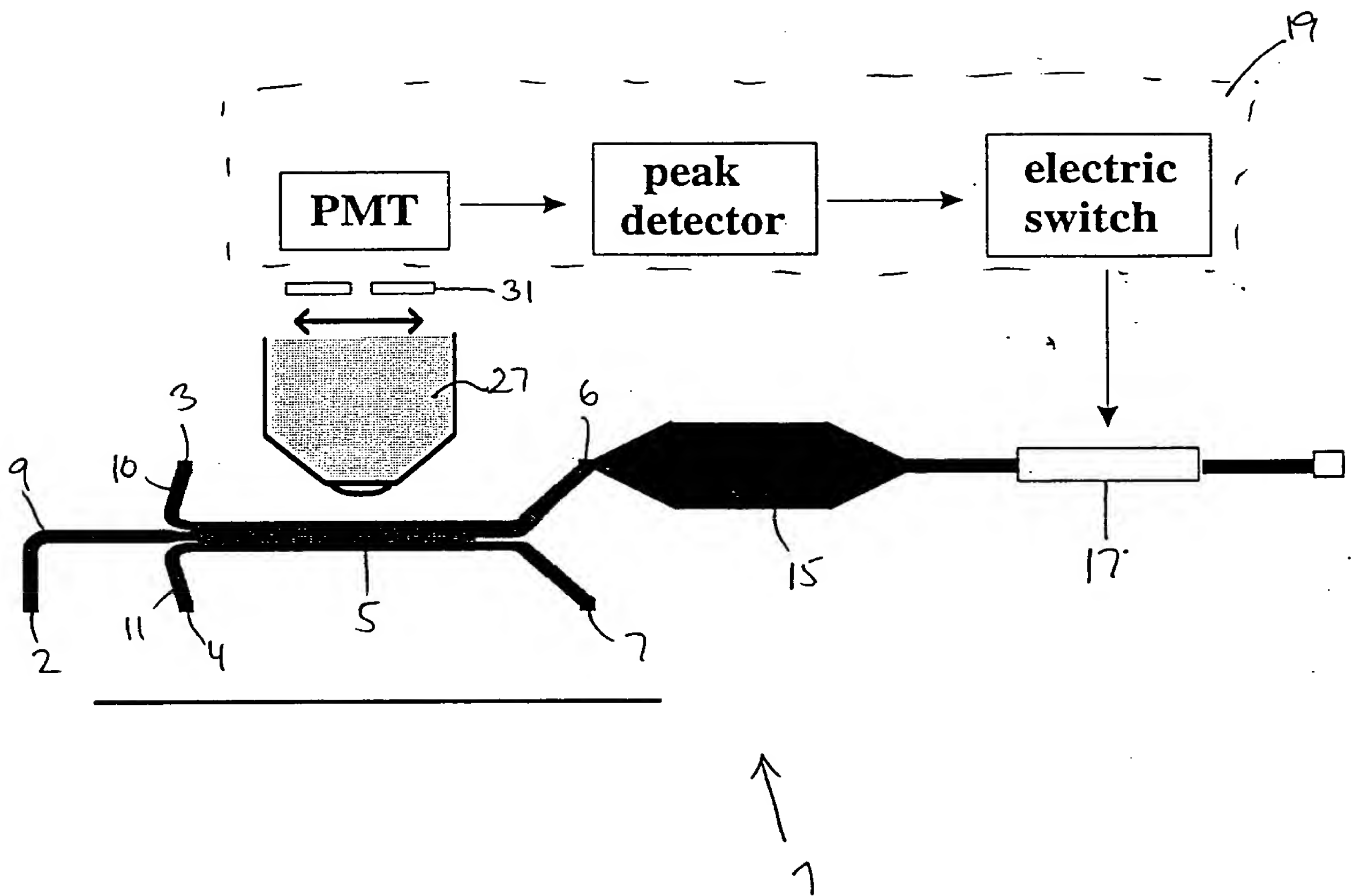


Fig. 8

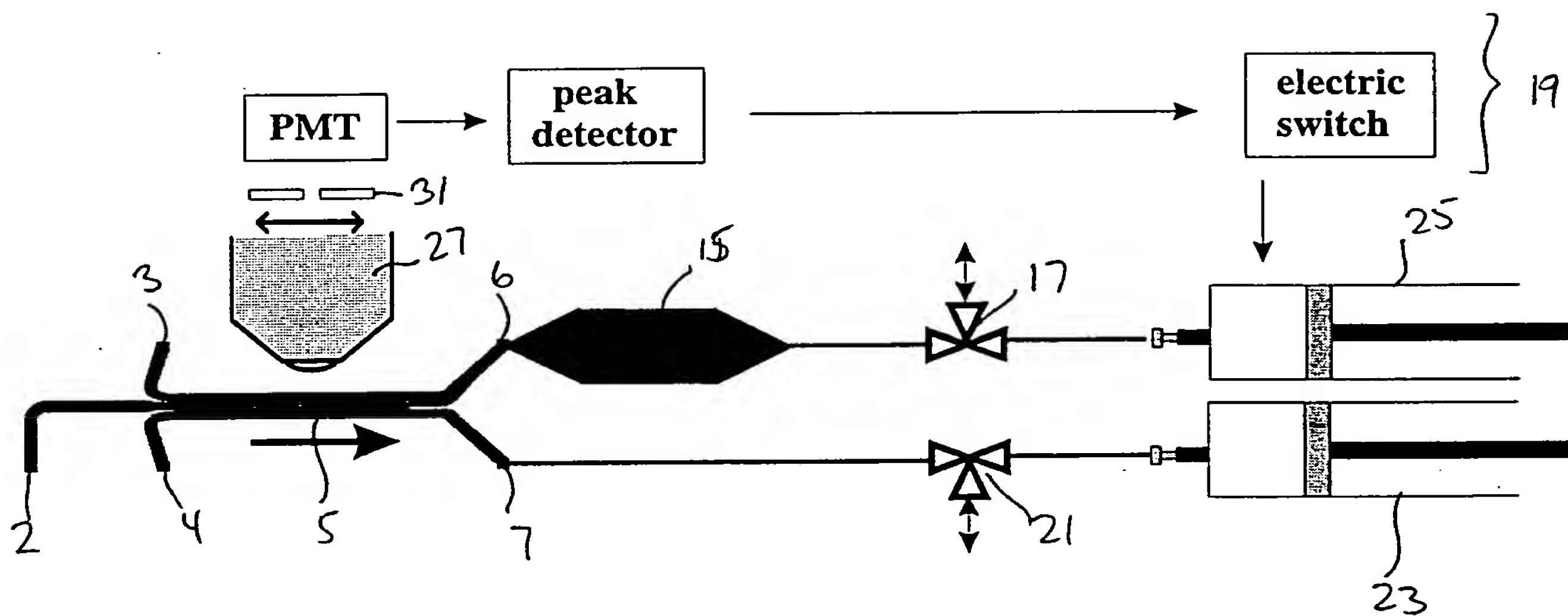


Fig. 9

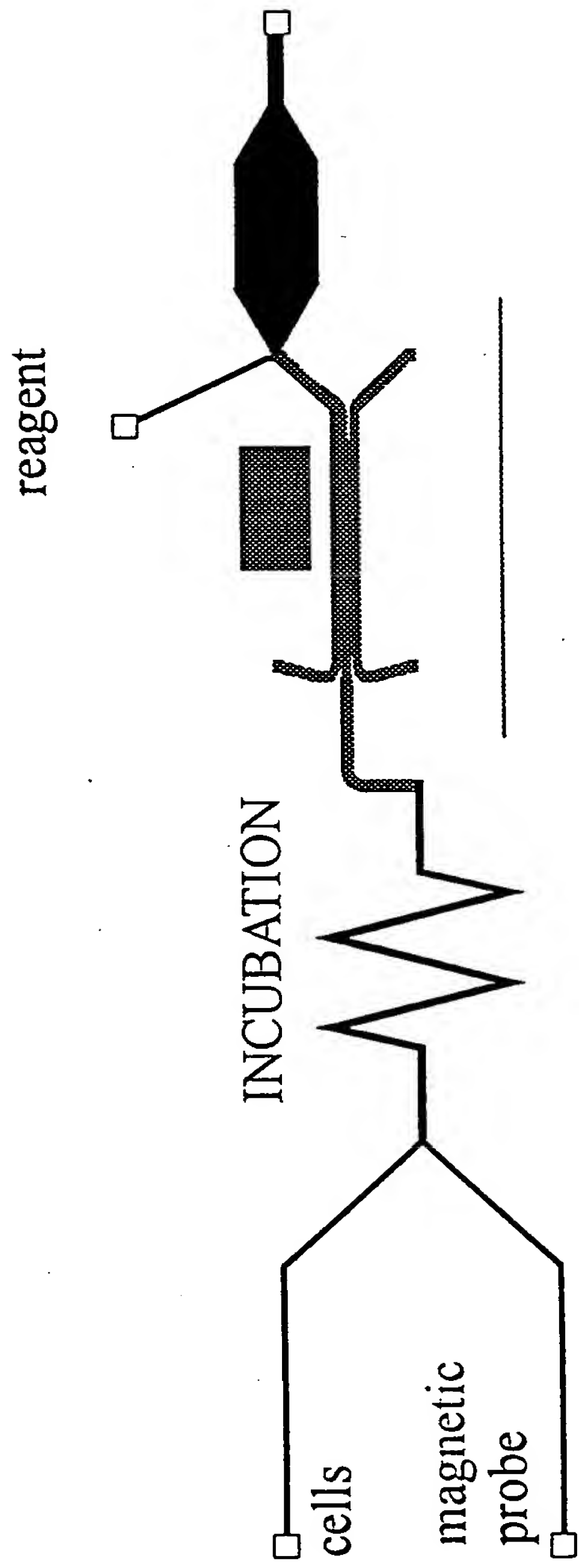
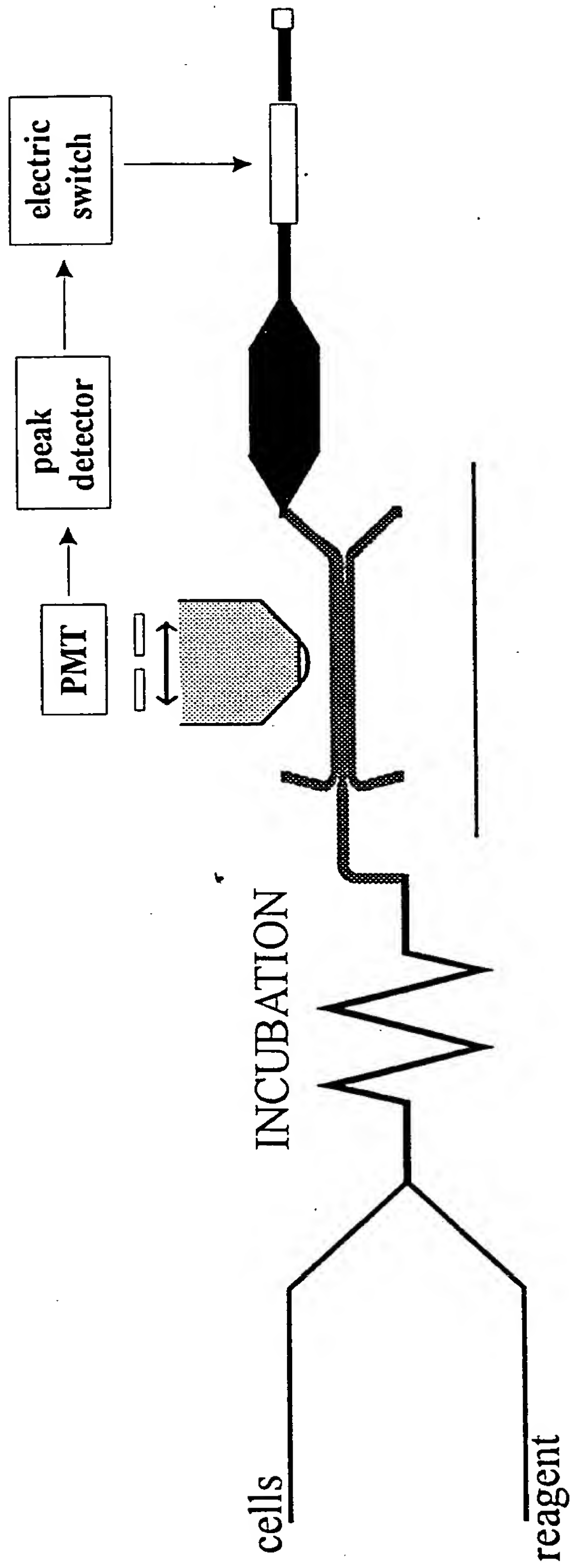


Fig. 10

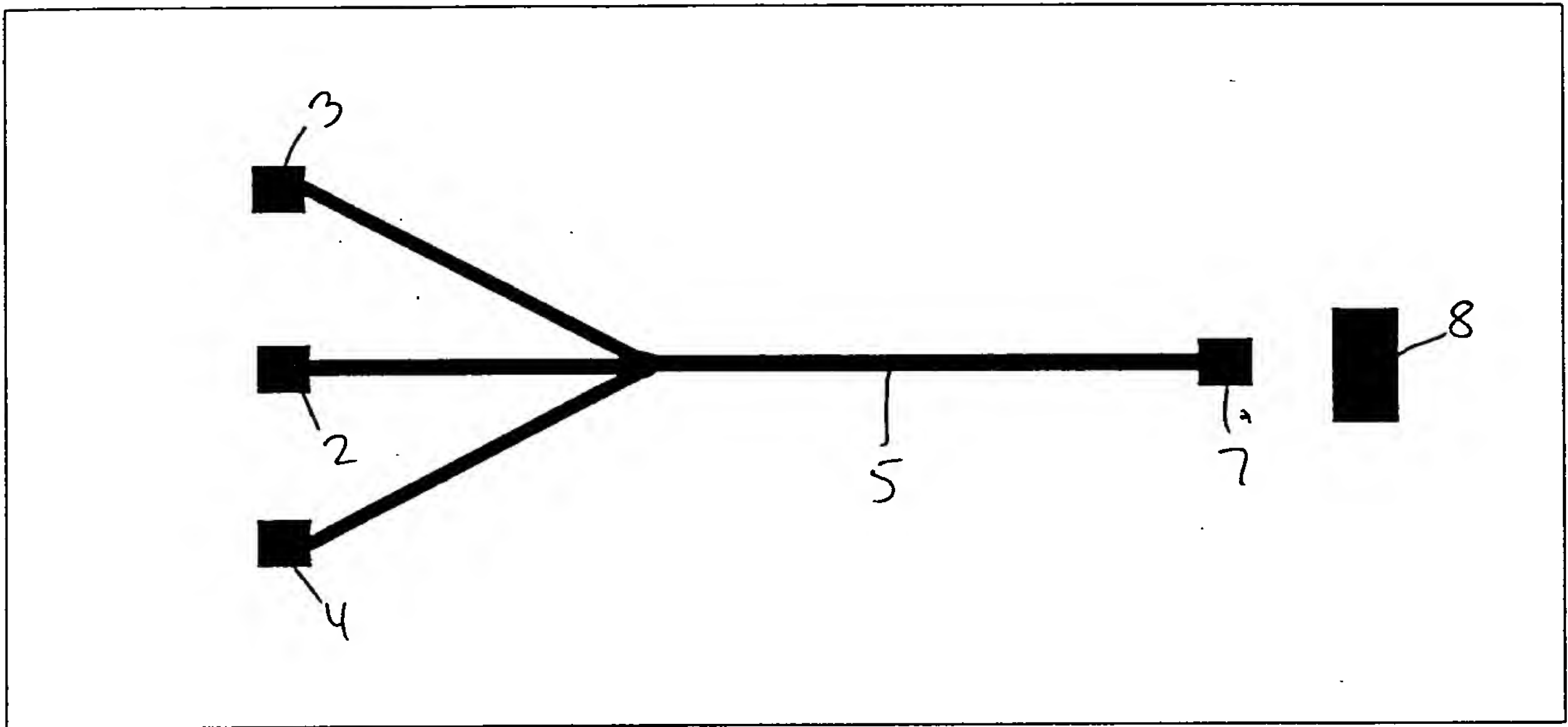
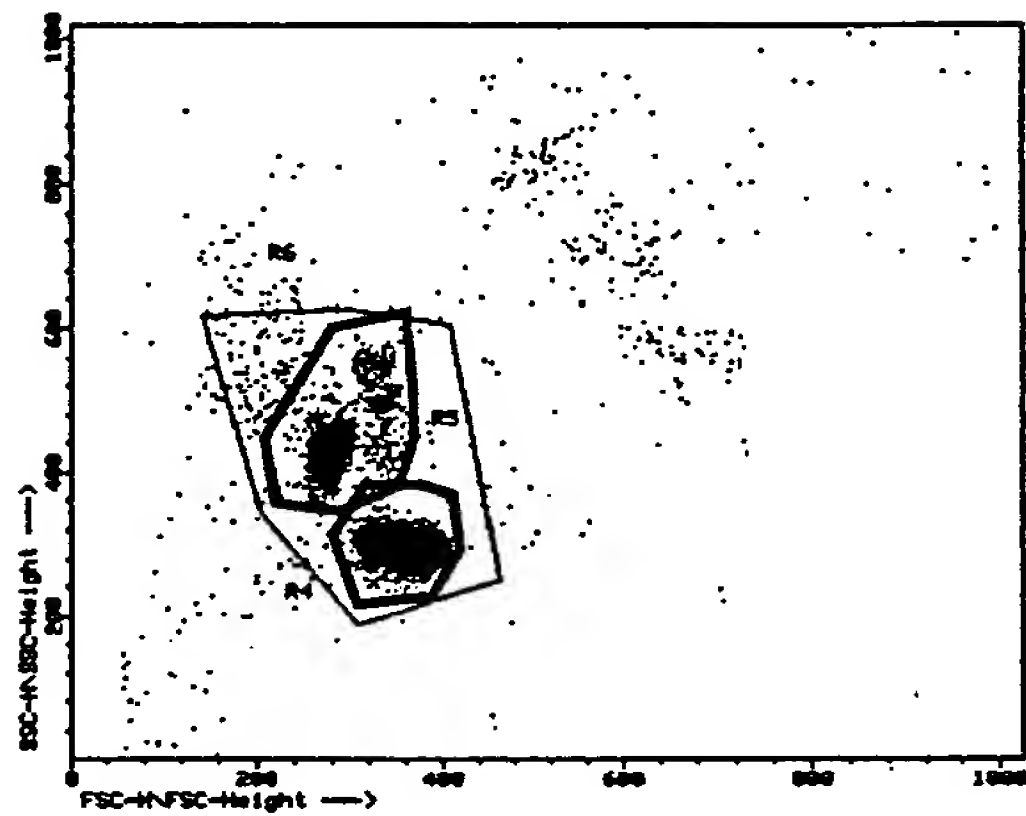
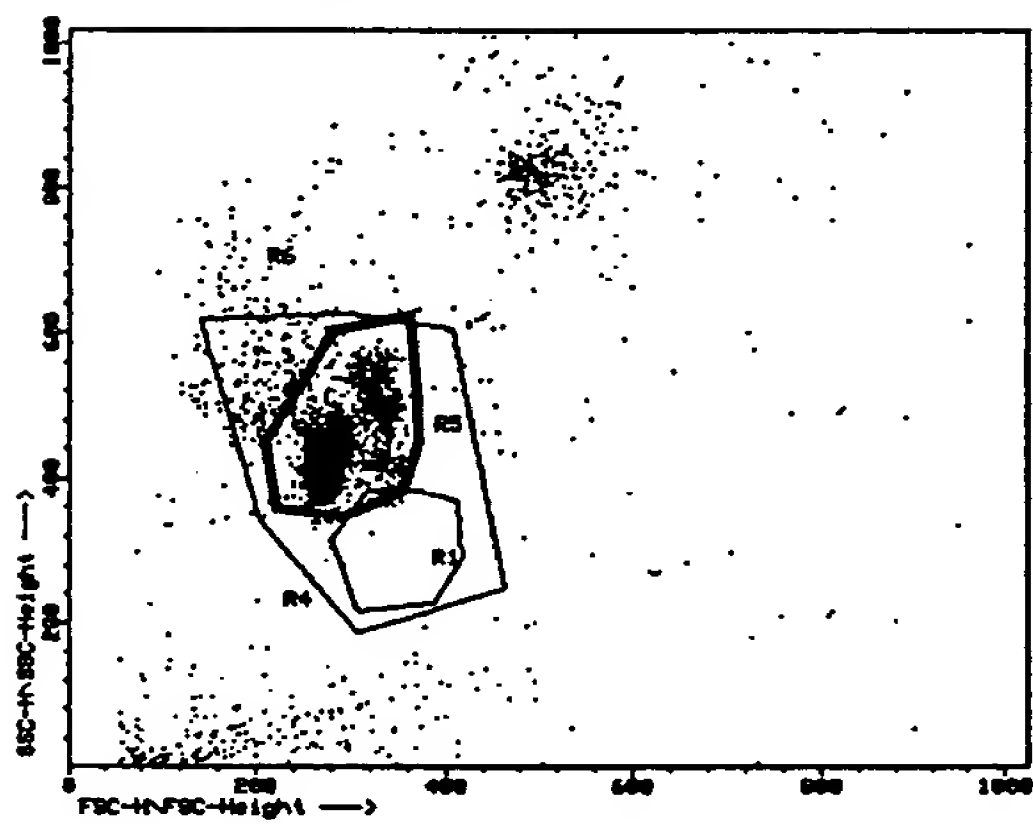


Fig. 11

(A) before sorting



(B) negative fraction



(C) positive fraction

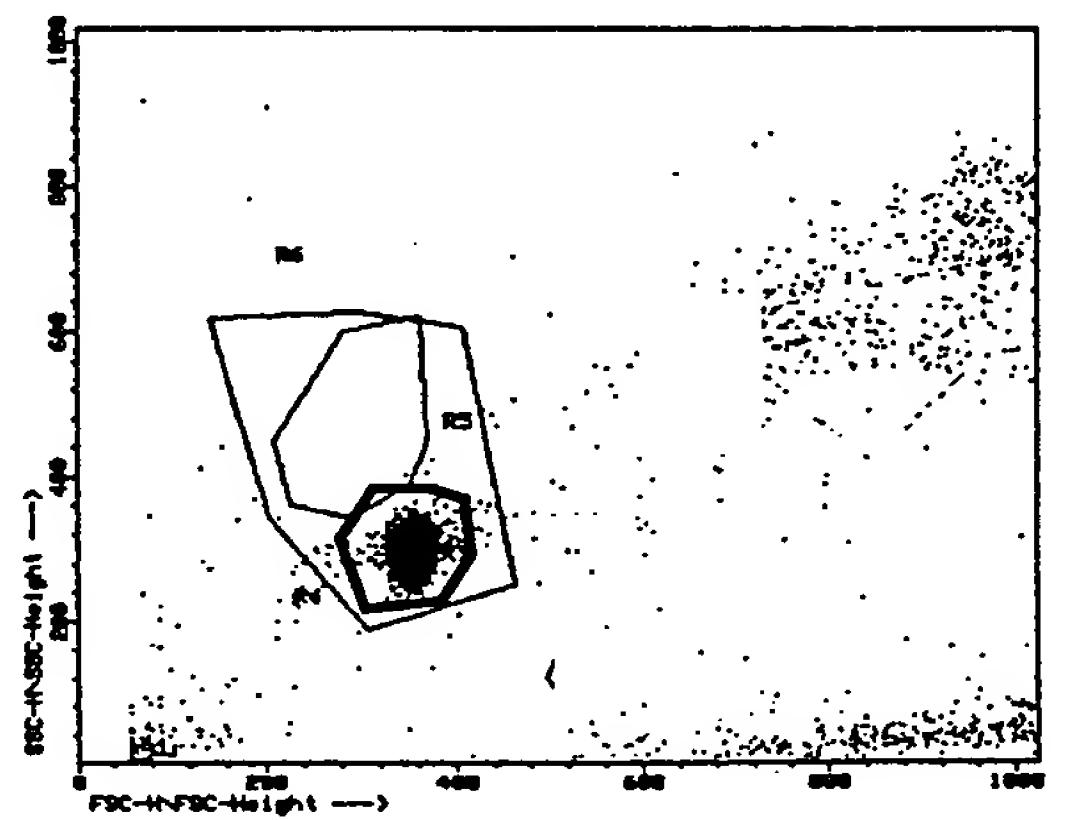


Fig. 12

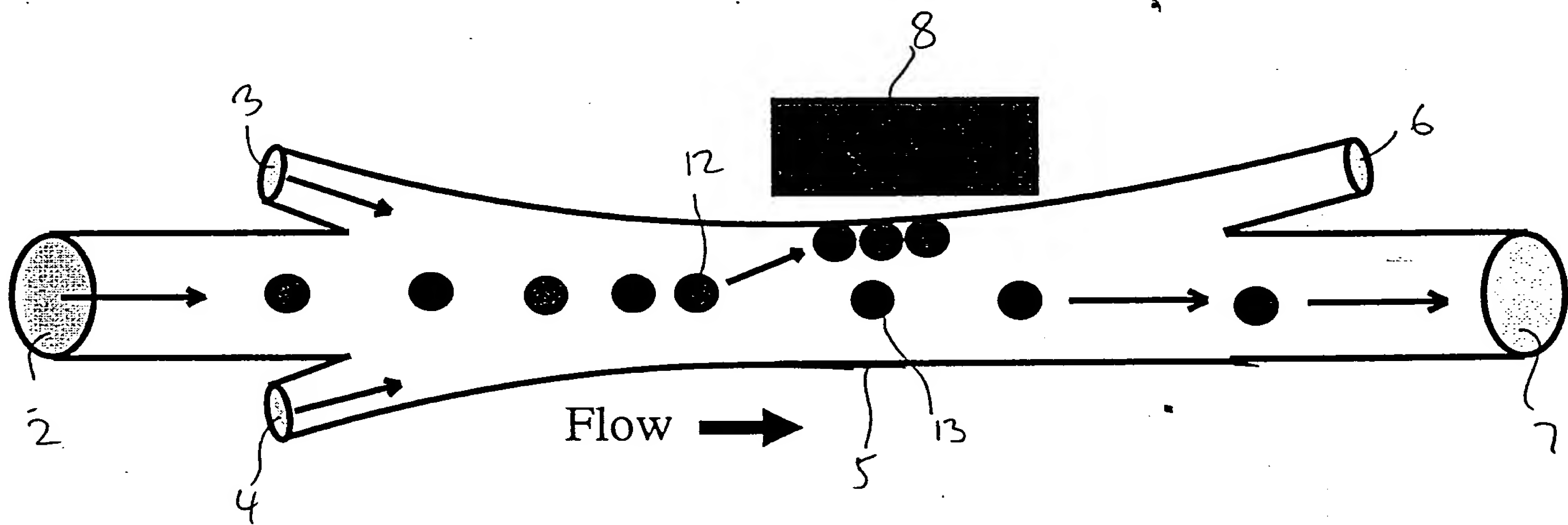


Fig. 13

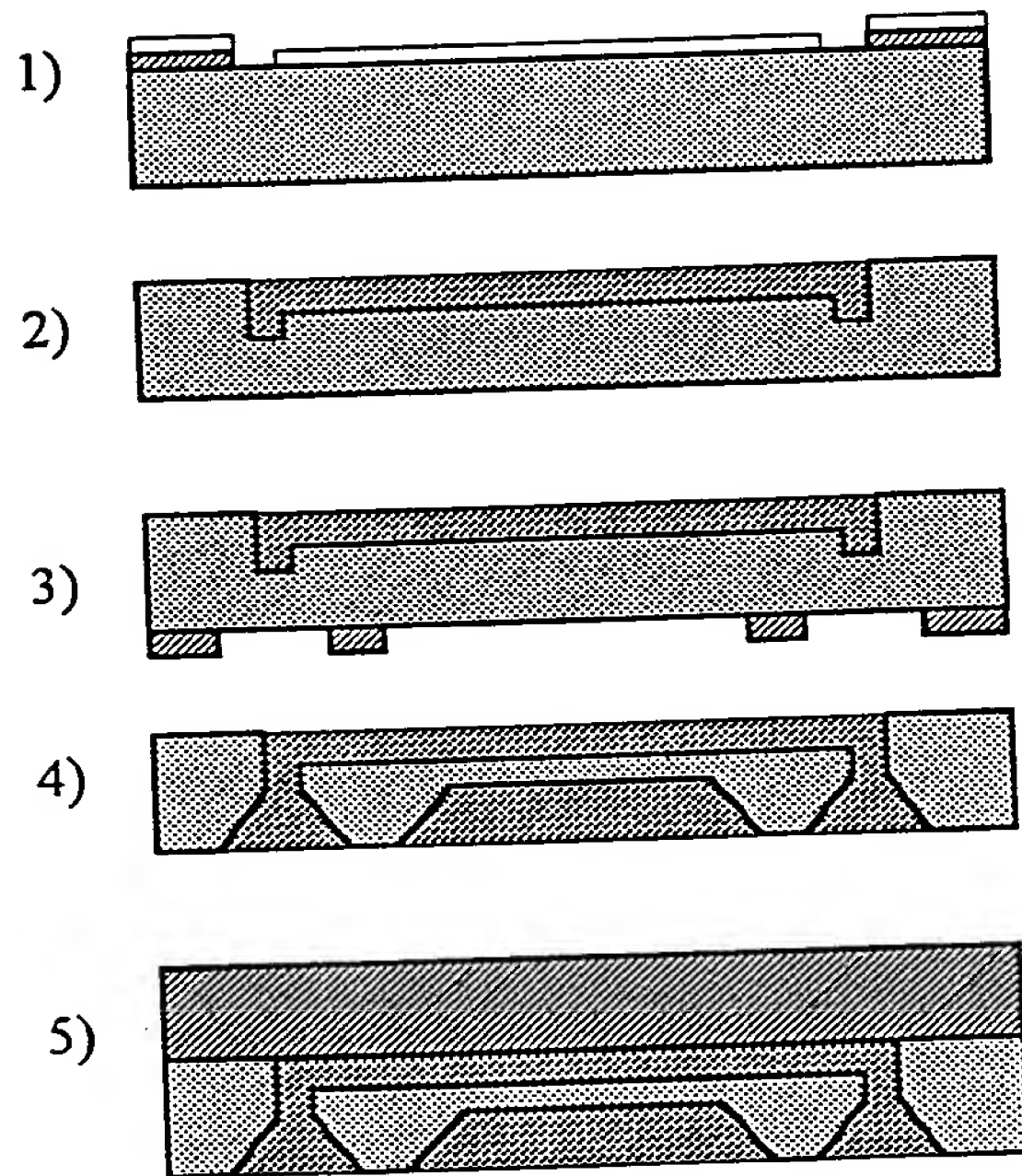


Fig. 14